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DETECTION OF LIMITING OR SURPLUS NUTRIENTS IN ALGAE

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Project WP 297

Detection of Limiting or Surplus Nutrients in Algae

George P. Fitzgerald

University of Wisconsin

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SUMMARY

The aim of this project has been to determine which inorganic nutrients required for the growth of algae can be stored in the algae in excess of their immediate requirements (luxury consumption) and develop techniques for the detection of such conditions.

Techniques have been developed for the detection of conditions of limiting or surplus nitrogen and phosphorus in algae and aquatic weeds. These tests have been evaluated with laboratory cultures and field populations and found to give meaningful results. They have been used to evaluate sources of nitrogen and phosphorus for the growth of algae and aquatic weeds in lakes.

This report presents three sections dealing with the evaluation and application of the techniques developed in this laboratory. In addition, the following current publications deal with studies supported in part by this grant:

1. Fitzgerald, G. P. The Biotic Relationships within Water Blooms. "ALGAE AND MAN," ed. by D. F. Jackson, Plenum Press (October 1964).

2. Lee, C. F., Clesceri, N. L. and Fitzgerald, G. P. "Studies on the Analysis of Phosphates in Algal Cultures" Int. J. Air Wat. Poll. 9, 715-722 (1965).

3. Fitzgerald, G. P. "Are Our Lakes Dying?" Highlights 1st Internat. Water Quality Symposium, Water Conditioning Assoc. Internat., Wheaton, Ill. p. 18-19 (1965).

4. Fitzgerald, G. P. and Nelson, T. C. "Extractive and Enzymatic Analyses for Limiting or Surplus Phosphorus in Algae" J. Phycology 2, 32-37 (1966).

5. Fitzgerald, G. P. and Faust, S. L. "Effect of Water Sample Preservation Methods on the Release of Phosphorus from Algae" Limn. Oceanogr. 12, 332-334 (1967).

6. Stewart, W. D. P., Fitzgerald, G. P. and Burris, R. H. "In Situ Studies on N_2-Fixation Using the Acetylene Reduction Technique" Proc. Nat. Acad. Sci., 58, 2071-2078 (1967).

7. Stewart, W. D. P., Fitzgerald, G. P. and Burris, R. H. "Acetylene reduction by nitrogen-fixing blue-green algae" Archiv fur Mikrobiologie 62:325-348 (1968).

8. Fitzgerald, G. P. "Detection of Limiting or Surplus Nitrogen in Algae and Aquatic Weeds" J. Phycol. 4, 121-126 (1968).

9. Fitzgerald, G. P. Environmental Requirements of Blue-Green Algae--Discussion. 1967, p. 97-102. Proc. of a Symposium, September 23-24, 1966. Fed. Water Poll. Control Admin., Corvallis, Oregon, 111 p.

It has been shown that the alkaline phosphatase activity of algae which are phosphorus-limited can be as much as 25 times greater than algae with sufficient phosphorus. In order to differentiate some of the factors involved when phosphorus-limited algae are placed in medium with adequate or surplus phosphorus the effect of adding PO_4-P to alkaline phosphatase analyses was compared to incubating algae in relatively high PO_4-P medium.

The green alga, Chlorella pyrenoidosa (Wis. 2005) cultured for seven days in Gorham's medium containing 0.2 mg P/L exhibited alkaline phosphatase activity indicative of phosphorus-limited growth conditions. Samples of algae (10 mg, dry weight) from this culture were washed into phosphorus-free medium and the enzymatic activity of the algal alkaline phosphatase measured over a one hour period in the presence of 0, 5, 10, or 20 mg PO_4-P/L. In addition, samples of algae were incubated during this same time and environment in media containing 0 or 20 mg PO_4-P/L and were then washed into phosphorus-free medium for alkaline phosphatase activity analyses.

The results of the analyses of alkaline phosphatase activity (Table 1) in the presence of 0, 5, 10, and 20 mg PO_4-P/L indicated relative enzymatic activities of 100, 35, 10 and 0%, respectively. Thus, concentrations of 5 mg PO_4-P/L or more will inhibit measurements of the activity of the alkaline phosphatase of 10 mg of Chlorella in a volume of 40 ml. In contrast, the alkaline phosphatase activity of algae incubated in the presence of 0 and 20 mg PO_4-P/L for one hour prior to the measurement of enzymatic activity in phosphorus-free medium were relatively 100 and 90%, respectively. Thus, the enzymatic activity of these algae had not been affected by such short-term incubation in relatively high phosphorus medium.

In another experiment the effect of added PO_4-P on phosphorus-limited algae was followed when phosphorus-limited Chlorella was added to Gorham's medium which contains 7 mg P/L. Analyses of suspended solids (dry weight), extractable PO_4-P from the algae and alkaline phosphatase activity of the algae were carried out over a period of nine days. The results of the analyses are presented in Figure 2.

Within two hours, the extractable PO_4-P had changed from 0.015 to 0.25 mg PO_4-P/100 mg algae (%P) and in five hours to 0.60% P, with no decrease in alkaline phosphatase activity. At 24 hours, the dry weight of the culture had increased from 110 to 145 mg/l, the extractable PO_4-P was 0.72% P, and there had been a slight decrease in enzyme activity from 2,700 to 2,400 units/mg. With increasing incubation times and culture growth, the extractable PO_4-P gradually decreased and the enzyme activity decreased. After six days of culture, P appeared to become limiting because the extractable PO_4-P had decreased to 0.08% P, and on the seventh and ninth days the enzyme activity increased.

These data indicate that the alkaline phosphatase activity of algae does not stop or decrease when algae are placed in medium containing adequate phosphorus for further growth. The activity of the enzyme must be reduced by growth (or dilution) under conditions of surplus substrate, much the same as in the case of the nitrogenase system of nitrogen-fixing, blue-green algae (Stewart, Fitzgerald and Burris 1968). Thus, aquatic plants which have been recently exposed to a source of relatively high concentration of available phosphorus could absorb enough phosphorus to indicate surplus phosphorus conditions by their total phosphorus content (Gerloff and Krombholz 1966) or extracted PO_4-P and still have high alkaline phosphatase activity. These conditions could possibly be used to advantage in ecological studies of the effects of slugs of nutrients or accidental spills in river systems since the relative rate of growth of plants could be followed by the time required for their enzymatic activity to decrease to its lowest level. The effects of different slugs of nutrients,

Field and Laboratory Evaluations of Bioassays for Nitrogen and Phosphorus with Algae and Aquatic Weeds[1)]

ABSTRACT

The rate of ammonia absorption in the dark and the amount of orthophosphate extracted by boiling water treatments have been used to follow the transient nature of the nitrogen and phosphorus nutrition of algae and aquatic weeds. The measurement of the enzymatic activity of algal alkaline phosphatase also used to follow phosphorus nutrition has been shown to be inhibited by the presence of 5 mg/L or more of PO_4-P, but the actual enzymatic activity of the alga is not inhibited by PO_4-P and is reduced only by growth (or dilution) under conditions of surplus available phosphorus. It is suggested that only terminal portions of aquatic weeds be used for nutritional bioassays because of nutritional differences between young and old portions of the same plant. Available nitrogen compounds related to rainfall have been shown to influence the nitrogen nutritional status of Spirogyra and Cladophora. In applications of bioassays the importance of testing each species of plants separately is shown by contrasting results obtained with nitrogen-fixing (phosphorus-limited) and non-fixing (nitrogen-limited) blue-green algae from the same environment.

INTRODUCTION

The reversal of the eutrophication of natural waters will depend upon limiting the available nutrients which are required for the growth of algae and aquatic weeds. The need for critical analyses for limiting plant nutrients in aquatic environments has been pointed out by several authors (Shapiro and Ribeiro 1965, Gerloff and Krombholz 1966, Oswald and Goluecke 1966, and Skulberg 1966). As Stewart, Fitzgerald and Burris (1967) have suggested, there is in particular a need for simple, inexpensive methods for studying the nutritional status of algae from a particular environment.

The present report is an evaluation of recently reported methods for detecting conditions of surplus or limiting nitrogen (Fitzgerald 1968) and phosphorus (Fitzgerald and Nelson 1966) in algae and aquatic weeds. The test for surplus or limiting nitrogen conditions is based upon the fact that algae or aquatic weeds which are limited by the supply of available nitrogen absorb ammonia nitrogen (NH_4-N) four to five times more rapidly in the dark than plants with surplus or adequate nitrogen and merely requires analyses of NH_4-N before and after incubation of the samples. The status of the phosphorus nutrition of algae or aquatic weeds can be evaluated or followed by measuring the amount of orthophosphate (PO_4-P) extracted when plants are killed by boiling water treatment or by determining the alkaline phosphatase (alkaline phosphomonoesterase) activity of the plants. Algae or aquatic weeds which had surplus phosphorus available for the amount of growth taken place will release more than 0.08 mg PO_4-P/100 mg algae after boiling water extraction. Plants which are limited in growth by the supply of available phosphorus contain little or no extractable PO_4-P and have as much as 25 times more alkaline phosphatase activity than plants with surplus available phosphorus. The measurement of alkaline phosphatase activity merely involves the incubation of plant material in the presence of a suitable substrate and the measurement of a colored end-product.

In addition to the methods for measuring or following the nitrogen and phosphorus nutritional status of algae or aquatic weeds which are dealt with here, it should also be pointed out that a simple, rapid, and inexpensive method of measuring in situ rates of

nitrogen fixation by planktonic and soil blue-green algae has been reported by Stewart, Fitzgerald and Burris (1967).

MATERIALS AND METHODS

The culture media used were modifications of Gorham's medium (Hughes et al., 1958). The details of the culture techniques for the algae and aquatic weeds used have been described previously (Fitzgerald and Nelson 1966, Fitzgerald 1968).

Analyses of PO_4-P in algal extracts was by the stannous chloride method and NH_4-N was analyzed by direct Nesslerization (Amer. Public Health Assn. 1965). All analyses were based on the dry weight of the algae or aquatic weeds used. The dry weight of planktonic algae could be measured on aliquot samples of algae filtered from the suspending medium and dried in tared vessels. The measurements of the dry weight of samples which could not be done by aliquot, such as Cladophora or Myriophyllum, were made with the actual samples used in the test by filtering and drying the quantity used after the enzymatic tests or PO_4-P extraction test were completed. Dry weights of samples extracted in boiling water for the PO_4-P extraction tests do not reflect the total dry weight of the original sample, but were considered accurate enough for the comparative purposes used here.

The extraction procedure used to detect the presence of surplus phosphorus in algae or aquatic weeds was to place 10-80 mg of washed (Gorham's minus P(-P) medium) plant material into 40 ml of Gorham's medium (minus P source; pH 7), extract in a boiling water bath for 50 minutes, centrifuge or remove plant material, and analyze the supernatant for orthophosphate. Alternative extraction methods using 5 minutes direct boiling or autoclaving techniques have not given as complete extraction results as 1 hour extractions in boiling water baths but, perhaps, could be of use on a comparative basis. Any extraction method which depends upon the killing of the plant tissue and allowing any PO_4-P present to leach to the supernatant would probably be sufficient as long as results could be correlated with those from the boiling water treatment (Fitzgerald and Faust 1967). The PO_4-P in the extracts was calculated as the mg P/100 mg (dry weight) plant material.

The alkaline phosphatase activity of the plant material was determined by suspending 1-20 mg of washed plant material in 32 ml Gorham's (minus P) medium, adding 4 ml of buffer solution (1 M Tris, 0.01 M MgCl, adjusted to pH 8.5 with acetic acid) and 4 ml of p-nitrophenylphosphate solution (30 mg/100 ml). The suspension was incubated at 35-37 C and the relative activity measured after 0.25-2 hr by removing 10 ml samples to centrifuge tubes containing 10 mg of orthophosphate P in 0.5 ml (to stop further enzyme activity). The tubes were centrifuged to obtain clear supernatants and the optical density measured at 395 mμ. An alternative method of alkaline phosphatase activity measurement would be the use of commercial enzymatic tablets, one brand of which (Phosphatabs-Alkaline Quantitative, Warner-Chilcott Labs., Morris Plains, N. J.) employs phenolphthalein phosphate and necessary co-factors. Data obtained with these prepared tablets have been found to be comparable to results with p-nitrophenylphosphate as substrate when tested with both algae and aquatic weeds. Activity was recorded as units of enzyme/mg (dry weight) of plant material in 40 ml. One unit of alkaline phosphatase is defined as the amount of enzyme which liberates 1 mμ mole of nitrophenol/hr under the prescribed conditions.

The comparative rates of NH_4-N absorption in the dark were used to differentiate between plant material from cultures containing surplus nitrogen versus cultures in which the growth of the plant material was limited by the available nitrogen. The tests were carried out by placing 5 to 20 mg (dry weight) of plant material, washed in nitrogen-free medium, into 10 to 30 ml of Gorham's medium (minus N) and adding 0.1 mg NH_4-N.

After 1 hour incubation at 25 C (+ 2) in the dark the NH_4-N content of the supernatant was compared to controls not containing plant material. In case the plant material is apt to settle during the incubation period, occasional mixing may be required to prevent local depletion of NH_4-N and erroneous rate results. The rates of NH_4-N absorption by samples with fourfold differences in weight will be equivalent on a dry weight basis if there has not been a local depletion of NH_4-N in the immediate environment of the plant materials. The results were calculated as the ug N absorbed/10 mg (dry weight)/hr.

RESULTS AND DISCUSSION

In order to demonstrate how measurements of the rate of NH_4-N absorption and the extraction of PO_4-P could be utilized to follow the transient nature of inorganic nutrition in plants, experiments were carried out with cultures of the aquatic weed, Ceratophyllum sp. Changes in the nitrogen and phosphorus nutrition of terminal two- to three-inch tips of this plant as samples were changed from high or low sources of nitrogen or phosphorus are presented in Figure 1 as the averages of triplicate analyses.

Samples of Ceratophyllum placed in Gorham's complete medium (solid line) absorbed phosphorus such that within six days the amount of extracted PO_4-P increased from the original amount of 0.14 to 0.56 mg PO_4-P/100 mg. When these samples were placed in medium without phosphorus (dotted line) for an additional 14 days the amount of PO_4-P extracted fell from 0.56 to 0.06 mg PO_4-P/100 mg. At that time a sample which was placed in Gorham's complete medium for eight days changed from being phosphorus-limited (0.06 mg PO_4-P/100 mg) to having surplus phosphorus (1.5 mg PO_4-P/100 mg). Samples of Ceratophyllum which were kept in the medium without phosphorus from the 20th to the 28th day were even further starved, the amount of PO_4-P extracted falling from 0.06 to 0.01 mg P/100 mg.

The rate of NH_4-N absorption of samples of Ceratophyllum placed in Gorham's complete medium (solid line) for six days fell from 11 to 5 ug NH_4-N/10 mg/hr, whereas the rate of samples in medium lacking nitrogen (dotted line) increased from 11 to about 18 ug NH_4-N/10 mg/hr, the range being 19 and 17 ug/hr. When these samples were reversed for 14 days, going from complete to medium lacking nitrogen and from medium lacking nitrogen to complete medium, the rates of NH_4-N absorption were reversed. On the 20th day part of the sample in the complete medium was placed in medium lacking nitrogen. After eight days, the rate of NH_4-N absorption of such samples was 14 ug N/10 mg/hr as compared to 9 ug N/10 mg/hr for samples remaining in complete medium.

These data indicate that the PO_4-P extraction technique and the rate of NH_4-N absorption technique can be used to follow changes in the phosphorus and nitrogen nutritonal status of this aquatic weed. The amounts of PO_4-P which can be extracted from the tip portions of this plant when it is placed in media with surplus phosphorus will, of course, depend upon the relative amounts of plant tissue and phosphorus available. In the experiments shown here relatively small portions (100-300 mg, dry weight) were planted in 1500 ml of Gorham's medium which contains 7 mg/L of phosphorus. In other experiments in which larger portions of plants were initially used the amounts of PO_4-P which could be extracted after suitable incubation in Gorham's medium were proportionately less. The tests of the rate of NH_4-N absorption tend to indicate that absorption rates of 15 ug NH_4-N/10 mg of plant/hr or higher represent a condition of nitrogen-limited growth for this plant. With increased culture in the presence of excess nitrogen, NH_4-N absorption rates of less than 5 ug NH_4-N/10 mg/hr would appear to be expected.

the conditions during the treatment, or conditions during the subsequent growth period could be relatively evaluated by a simple enzymatic assay.

The relative changes in the total nitrogen content and the rate of NH_4-N absorption which take place when algae are transferred from relatively high to limiting nitrogen conditions or vice versa have been reported (Fitzgerald 1958) using Chlorella pyrenoidosa. Chlorella originally from nitrogen-limited cultures contained only 2.5% N and had an NH_4-N absorption rate of approximately 40 ug N/10 mg/hr, whereas Chlorella from Gorham's complete medium had a total nitrogen content of 7.3% and an NH_4-N absorption rate of 14 ug N/10 mg/hr. With increasing time of incubation of the originally nitrogen-limited Chlorella in complete medium the total nitrogen content increased such that at 22 hours it had increased from 2.5 to 5.9% N and the rate of NH_4-N absorption had decreased from 40 to 5 ug N/10 mg/hr. Chlorella from complete medium when placed in nitrogen-free medium exhibited a gradual increase in the NH_4-N absorption rate from 14 to 45 ug N/10 mg/hr after 22 hours. However, Chlorella from the complete medium was not able to grow enough in the nitrogen-free medium within 22 hours to bring about a significant decrease in the percentage of total nitrogen. Therefore, changes in the nitrogen nutrition of algae can be followed by the changes in the rate of absorption of NH_4-N in the dark before the alga can grow enough to change its chemical composition. Changes in the rate of NH_4-N absorption are dependent upon the nitrogen nutrition of the algae at the time of sampling, whereas the total nitrogen content is dependent upon further growth in the case of algae exposed to adequate and then to limited nitrogen conditions.

The effect of which portion of a plant is used for nutritional analyses can be of importance in some instances. When the average rate of NH_4-N absorption of seven samples of terminal tips of Cladophora filaments from Lake Mendota was compared to the average of 10 samples of more basal sections of filaments no difference could be detected. However, when analyses of leaves from within eight inches of the tips of sprigs of Myriophyllum sp. from Lakes Wingra and Mendota were compared with those of older leaves from more basal positions, there were very obvious differences noted. The rate of NH_4-N absorption by young leaves (14-15 ug N/10 mg/hr) indicated they were probably nitrogen deficient, whereas the amount of PO_4-P extracted from them indicated they had surplus phosphorus present (0.19-0.20 mg PO_4-P/100 mg). Older leaves appeared to be less nitrogen deficient (8 ug NH_4-N absorbed/10 mg/hr) but were phosphorus limited (0.05 to 0.07 mg PO_4-P extracted/100 mg). Thus, analyses made on total plants or even on all the plant above the mud line may give very confusing results because one portion may have surplus phosphorus and the other may be limited in phosphorus and have relatively high levels of alkaline phosphatase activity. One must be aware of the basic patterns of the mobilization of nutrients in a particular plant species or for a particular nutrient when dealing with such complex situations. It is suggested that only terminal portions of plants be used for comparative analyses until more information on the mobility of nutrients is generally available.

The nutrients in rainfall have been reviewed by Putnam and Olson (1960), Mackenthun and Ingram (1967) and Fruh (1967). In studies using the rate of NH_4-N absorption in the dark by algae, we found that there was a dramatic effect by the nitrogen related to rainfall on the results obtained with the green algae, Spirogyra sp. and Cladophora sp. Samples of Spirogyra obtained on June 7, 1957, from the surface of a small gravel-lined pond near Madison, Wisconsin, (Salmo Pond) were pale, yellowish green, compared to dark green samples of Spirogyra obtained from a nearby eutrophic creek (Black Earth Creek). The rates of NH_4-N absorption of the samples were 16 and 8 ug N/10 mg/hr, respectively. After a rainfall (0.4 inches) the night of June 7, samples of Spirogyra collected from the pond on June 8 were bright green, and the rate of NH_4-N absorption had fallen to 8 ug N/10 mg/hr. Samples of Spirogyra from Black Earth Creek obtained after the rain were not

affected by the rain, their absorption rate remaining at 8 ug N/10 mg/hr. Similarly, 11 samples of Cladophora from the rocks along the shoreline of Lake Mendota collected and tested during a dry period, June 1 to 5, 1967, had an average NH_4-N absorption rate of 13 ug N/10 mg/hr. Between June 7 and June 10, the area received over two inches of rain. Eight samples of Cladophora collected on June 10 and 11 had an average NH_4-N absorption rate of 5 ug N/10 mg/hr. In the later part of July, after a week or more with no rain, 11 samples of Cladophora from Lake Mendota shoreline had an average NH_4-N absorption rate of 25 ug N/10 mg/hr, whereas two days later, after a considerable rain (0.55 inches) the average of nine samples was 9 ug N/10 mg/hr.

These data indicate that there are available nitrogen compounds related to rainfall which can influence the nitrogen nutrition of algae in natural environments. That the effect is due to nutrients in the rainfall and not due to nutrients washed into the lakes or ponds has not been differentiated.

The effect of the immediate environment on the nitrogen nutrition of algae is demonstrated by the facts that six samples of the green alga, Hydrodictyon sp., collected from the surface of a small pond near University Bay, Lake Mendota, on July 11 and 12, 1967, had an average NH_4-N absorption rate of 44 ug N/10 mg/hr, whereas six samples of the same alga collected from a small shaded outlet stream, which also received underground discharges from a nearby irrigation project, absorbed little or no NH_4-N during one hour in the dark. Therefore, one can conclude that the Hydrodictyon from the pond surface was nitrogen limited, whereas the Hydrodictyon from the outlet stream had sufficient or surplus available nitrogen. Samples of Hydrodictyon collected on July 14 from the surface of the pond were found to have small green spots among the network of Hydrodictyon cells. These green spots were a species of Nostoc and samples of it were found to be capable of active nitrogen fixation. The NH_4-N absorption rate of the Hydrodictyon after it was cleaned of the Nostoc was found to have fallen from the previous rate of 44 ug N/10 mg/hr to 10 ug N/10 mg/hr. It was assumed that this decrease in NH_4-N absorption rate was due to the effect of combined nitrogen obtained from the nitrogen-fixing Nostoc in its immediate environment, as has been shown to be possible by Stewart (1967) and Jones and Stewart (1968) in which cases Nostoc and Calothrix supplied combined nitrogen to higher plants, non-nitrogen-fixing algae, fungi and bacteria, which grew through layers of surface soil rich in the nitrogen-fixing algae or in close association with them.

The influence of nitrogen-fixing algae on the nitrogen nutrition of the phytoplankton and aquatic weeds of an environment has been the subject of much conjecture by many biologists. In order to determine if nitrogen-fixing, bloom-forming, blue-green algae would contribute available nitrogen to other bloom-forming algae in the immediate environment (mixed blooms), samples of each were analyzed (Table 2). On two separate occasions during 1967, Lake Monona (Madison, Wisconsin) contained mixed blooms of Microcystis sp. and Aphanizomenon sp. such that clumps of either alga could be collected separate from the other. Measurements of the rate of NH_4-N absorption by the Aphanizomenon samples indicated six or less ug N were absorbed/10 mg/hr whereas the Microcystis samples had rates of 20-23 ug N/10 mg/hr. It was assumed that the Aphanizomenon (a nitrogen-fixing alga) was not nitrogen-limited and that the Microcystis was nitrogen-limited. Analyses of the algae for surplus phosphorus by measuring the amount of PO_4-P extracted by boiling water indicated that the Aphanizomenon was probably phosphorus limited (0.10 mg P extracted/100 mg algae), whereas the Microcystis contained surplus phosphorus (0.42 mg P/100 mg algae). Analyses carried out in 1965 on mixed blooms from Lake Monona of Microcystis sp. and Anabaena sp. indicated that the Microcystis had surplus available phosphorus (0.22 mg PO_4-P extracted/100 mg algae; 2,200 units alkaline phosphatase/mg (p-nitrophenylphosphate test): 0.4 units alkaline phosphatase/mg (Phosphatabs test)),

whereas the Anabaena was P-limited (0.06 mg PO_4-P extracted/100 mg algae; 18,000 units alkaline phosphatase/mg (p-nitrophenylphosphate test); 20 units alkaline phosphatase/mg (Phosphatabs test)). Thus, it can be assumed that the Aphanizomenon and Anabaena had sufficient or surplus nitrogen which was not available to the Microcystis and the Microcystis had surplus phosphorus which was not available to the Aphanizomenon and Anabaena. A further assumption can be made: the sources of nitrogen and phosphorus for these algae must have been other than the environment of the blooms, possibly bottom deposits, since the Microcystis had run out of available nitrogen but still had surplus phosphorus and the Aphanizomenon and Anabaena had run out of available phosphorus. If both species absorbed surplus nutrients from bottom deposits and grew in the photic zone until starved by lack of some nutrient, such situations would occur. Sufficient data on the chemistry of lake surface waters have not been accumulated to determine if the algal nutrients in the surface waters change as blooms of algae develop and decline or if algae such as Microcystis can absorb available phosphorus compounds at the expense of nitogen-fixing algae, such as Aphanizomenon or Anabaena.

CONCLUSIONS

The studies reported here demonstrate the usefulness of simple bioassay procedures for the nitrogen and phosphorus nutritional status of algae and aquatic weeds. These procedures are supplemented by the rapid and simple procedure for determining the rate of nitrogen-fixation by blue-green algae reported by Stewart, Fitzgerald and Burris (1967).

The characteristics of each type of analysis for the nutritional status of algae or aquatic weeds make it possible to obtain valuable ecological information by comparing results from different types of tests. It has been pointed out how recent phosphate fertilization of an environment can be assumed from data showing high extractable PO_4-P and high alkaline phosphatase activity. Similarly, high total nitrogen analyses in combination with high NH_4-N absorption rates tend to indicate that the plant material has recently been exposed to comparatively less available nitrogen than in the recent past.

The effect of the local environment or recent changes in the environment on the nutrition of algae or aquatic weeds has been discussed. It must be emphasized that local environments will control the nutritonal status of plants and certain factors, such as recent rains or unusual circulation patterns in lakes which cause changes in the sources of algae (epilimnion versus thermocline), should be taken into account when interpreting the results of nutritional tests.

It is of utmost importance to describe the portion of the plant or species of algae which has been analyzed. Mixed algal samples are of no more value than total plant analyses since different results can be obtained from each type of species or parts of a plant. The data presented have indicated that non-nitrogen-fixing algae can be limited by available nitrogen in the same environment in which nitrogen-fixing algae are limited by available phosphorus. Thus, one must consider the various types of plankton or aquatic weeds from each local environment on a separate basis and make very few total environmental predictions unless all situations have been evaluated.

ACKNOWLEDGMENTS

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TABLE 1

The Effect of PO_4-P on Measurements of Alkaline Phosphatase Activity*

PO_4-P Treatment (mg/L)	Relative Phosphatase Activity (%)	
	Analysis During PO_4-P Treatment	Analysis After PO_4-P Treatment
0	100	100
5	35	--
10	10	--
20	0	90

*Chlorella pyrenoidosa (Wis. 2005) 10 mg algae/40 ml

TAPLE 2. Comparisons of the nitrogen and phosphorus nutritional status of algae from mixed blooms in Lake Monona

	Samples	Rate of NH_4-N Absorbed (ug N/10 mg)	PO_4-P Extracted (mg PO_4-P/100 mg)	Phosphate Test (units/mg)	Phosphatabs Test (units/mg)
1967	Microcystis sp.	20-23	0.42	---	---
	Aphanizomenon sp.	5 or less	0.10	---	---
1966	Microcystis sp.	---	0.22	2,200	0.4
	Anabaena sp.	---	0.05	18,000	20

Figure 2

Relationship between growth, extracted PO_4-P, and alkaline phosphatase activity of phosphorus-limited Chlorella pyrenoidosa (Wis. 2005) when placed in a medium containing 7 mg P/L.

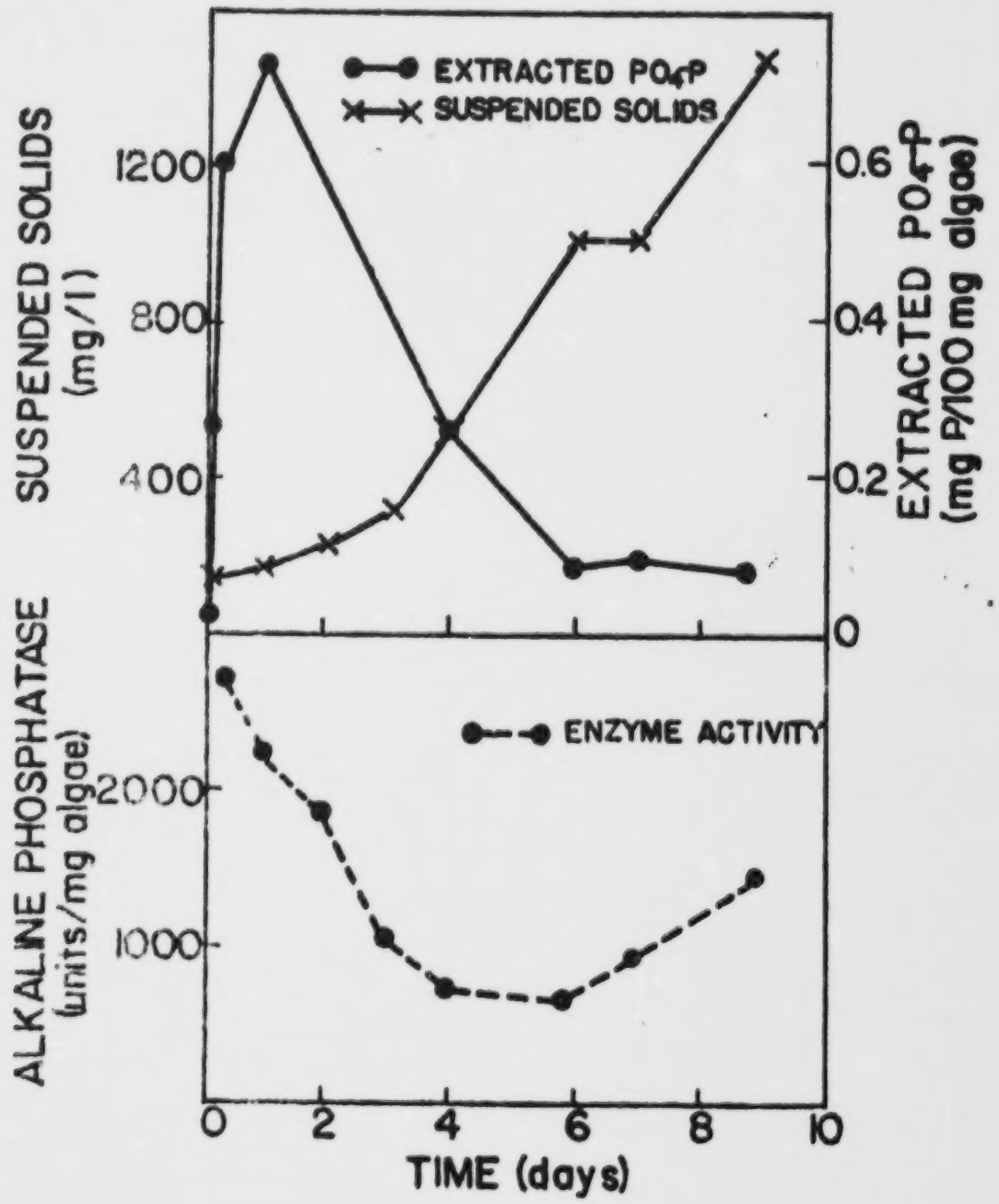
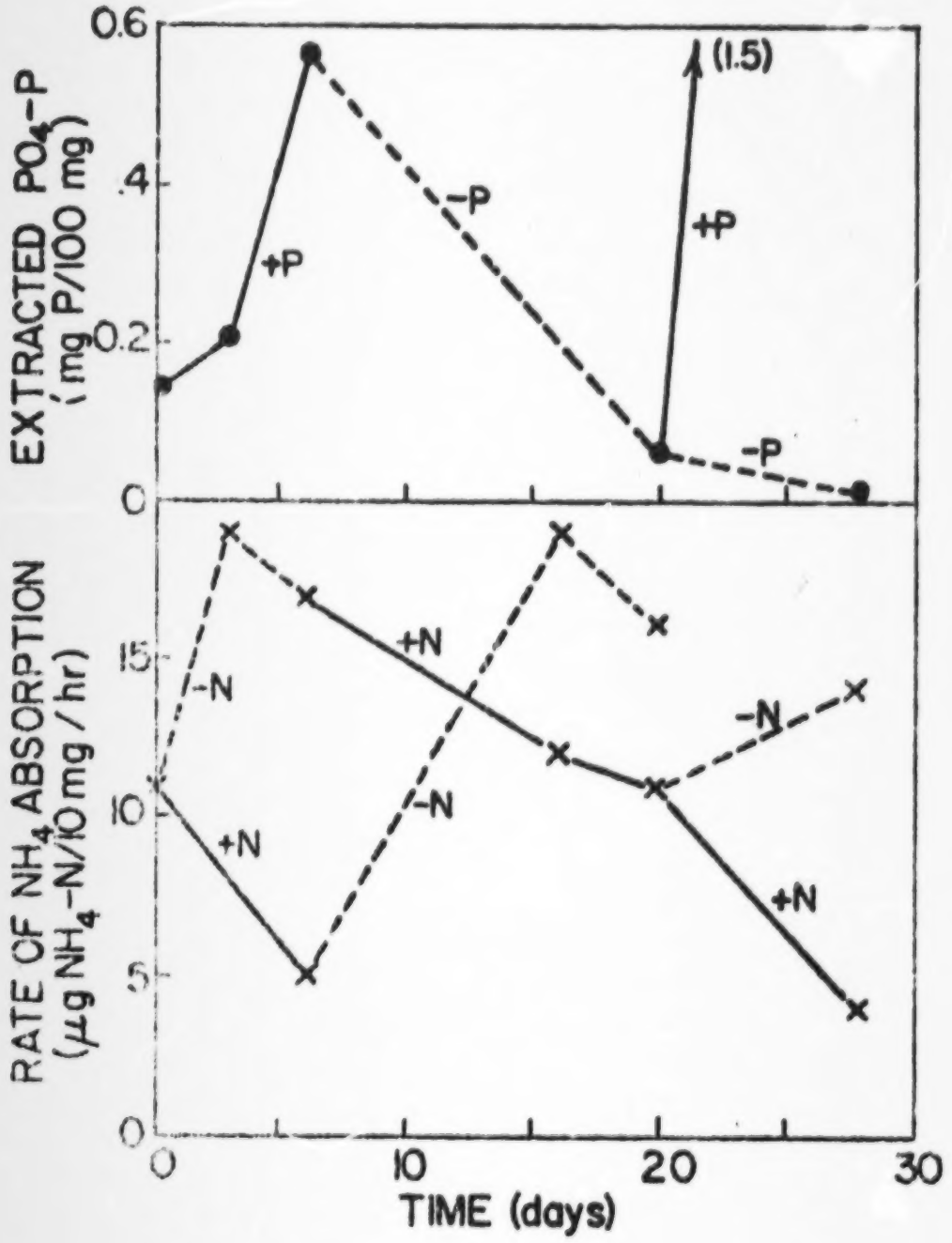


Figure 1

PO_4-P extracted and rate of NH_4-N absorption of tips of Ceratophyllum sp. when plants were incubated in Gorham's complete medium (solid lines) or media lacking nitrogen or phosphorus (dotted lines).



Nutrient Sources for Algae and Aquatic Weeds

SUMMARY

Simple bioassays for limiting or surplus nitrogen and phosphorus conditions in algae or aquatic weeds have been used to follow the nutrition of algae and aquatic weeds during the summer of 1968. Of particular interest has been the sources of nitrogen and phosphorus available to these plants. It has been shown that during the summer rain could be the major source of available nitrogen to Cladophora sp. in Lake Mendota. Increases in phosphorus associated with rainfalls were detected, but were not as dramatic as increases in nitrogen. In addition, studies have shown that mixed blooms of planktonic algae do not share their nutrients with other algae even when one species may have a surplus and another be nutrient-limited. Comparisons of surface and subsurface phytoplankton have indicated that at certain times surface plankton can be nutrient-limited while the same species from subsurface sources had adequate or surplus nutrients. Fall overturn as a nutrient source has been used to demonstrate how similar changes can take place in the nutrition of different types of algae. The results of these studies are used to point out some of the important factors involved in field studies of the nutrition of aquatic plants.

INTRODUCTION

The use of simple bioassay procedures for detecting the nutritional status of algae and aquatic weeds at the time of sampling appears to be a logical method for evaluating nutrient sources for algae and aquatic weeds in situ. In order to demonstrate how such tests could be of value we have followed nutritional changes in a green alga, Cladophora sp., which grows attached to rocks in surface waters and could be expected to reflect changes in the nutrient content of the surface waters. Sources of nutrients which affected the algae which grow at the surface could be evaluated and correlated with information from the literature on nutrient sources and their implication in the eutrophication of lakes. Changes in the nutritional status of other attached and planktonic algae or aquatic weeds were used to demonstrate some of the contrasting factors involved in the nutrition of different planktonic algae or planktonic algae versus attached plants.

The basis of the tests for limiting or surplus nutrients in algae and aquatic weeds have been discussed previously (Fitzgerald and Nelson 1966; Fitzgerald 1968a, Fitzgerald 1968b). In addition, a simple test for measuring the rate of nitrogen fixation by blue-green algae has been published (Stewart, Fitzgerald and Burris 1967, 1968). These tests are based on analyses of particular chemical fractions or enzymatic reactions which reflect limiting or surplus conditions of a nutrient more meaningfully than do total plant analyses. Algae or aquatic weeds growing under conditions in which their growth is limited by the amount of available phosphorus have little or no extractable PO_4-P, but relatively high alkaline phosphatase (alkaline phosphomonesterase) activity. Algae or aquatic weeds limited by available nitrogen absorb NH_4-N in the dark four to five times faster than algae or aquatic weeds with adequate or surplus available nitrogen.

MATERIALS AND METHODS

The extraction procedure used to detect the presence of surplus phosphorus in algae or aquatic weeds was to place 10 to 80 mg of plant material washed in Gorham's minus P (-P) medium (Hughes et al. 1958) into 40 ml of Gorham's medium (minus P source; pH 7), extract in a boiling water bath for 30 minutes, centrifuge or remove plant material, and analyze the supernatant for orthophosphate. Alternative extraction methods using 5 minutes direct

boiling or autoclaving techniques have not given as complete extraction results as one hour extractions in boiling water baths but, perhaps, could be of use on a comparative basis. Any extraction method which depends upon the killing of the plant tissue and allowing any PO_4-P present to leach to the supernatant would probably be sufficient as long as results could be correlated with those from the boiling-water treatment (Fitzgerald and Faust 1967). The PO_4-P in the extracts was calculated as the mg P/100 mg (dry weight) plant material.

The alkaline phosphatase activity of the plant material was determined by suspending 1 to 20 mg of washed plant material in 32 ml Gorham's (minus P) medium, adding 4 ml of buffer solution (1 M Tris, 0.01 M MgCl, adjusted to pH 8.5 with acetic acid) and 4 ml of p-nitrophenylphosphate solution (30 mg/100 ml). The suspension was incubated at 35 to 37 C and the relative activity measured after 0.25 to 2 hr by removing 10 ml samples to centrifuge tubes containing 10 mg of PO_4-P in 0.5 ml (to stop further enzyme activity). The tubes were centrifuged to obtain clear supernatants and the optical density measured at 395 mμ. An alternative method of alkaline phosphatase activity measurement would be the use of commercial enzymatic tablets, one brand of which ('Phosphatabs-Alkaline Quantitative, Warner-Chilcott Labs., Morris Plains, N. J.) employs phenolphthalein phosphate and necessary co-factors. Data obtained with these prepared tablets have been found to be comparable to results with p-nitrophenylphosphate as substrate when tested with both algae and aquatic weeds. Activity was recorded as units of enzyme/mg (dry weight) of plant material in 40 ml. One unit of alkaline phosphatase is defined as the amount of enzyme which liberates 1 mμ mole of nitrophenol/hr under the prescribed conditions.

Measurements of comparative rates of NH_4-N absorption in the dark were carried out by placing 5 to 20 mg (dry weight) of plant material, washed in nitrogen-free medium, into 10 to 30 ml of Gorham's medium (minus N) and adding 0.1 mg NH_4-N. After 1 hour incubation at 25 C (+ 2) in the dark the NH_4-N content of the supernatant was compared to controls not containing plant material. In case the plant material is apt to settle during the incubation period, occasional mixing may be required to prevent local depletion of NH_4-N and erroneous rate results. The rates of NH_4-N absorption by samples with fourfold differences in weight will be equivalent on a dry weight basis if there has not been a local depletion of NH_4-N in the immediate environment of the plant materials. The results were calculated as the μg N absorbed/10 mg (dry weight)/hr.

Analyses of PO_4-P in algal extracts was by the stannous chloride method and NH_4-N was analyzed by direct Nesslerization (Amer. Public Health Assn. 1965). All analyses were based on the dry weight of the algae or aquatic weeds used. The dry weight of planktonic algae could be measured on aliquot samples of algae filtered from the suspending medium and dried in tared vessels. The measurements of the dry weight of samples which could not be done by aliquot, such as Cladophora or Myriophyllum, were made with the actual samples used in the test by filtering and drying the quantity used after the enzymatic tests or PO_4-P extractions were completed. Dry weights of samples extracted in boiling water for the PO_4-P extraction tests do not reflect the total dry weight of the original sample, but were considered accurate enough for the comparative purposes used here.

The algae of interest in mixed blooms were separated by the use of plankton netting (silk bolting cloth) of different meshes, different floatation characteristics, or simple picking if all else failed. A sample listed as Microcystis frequently also contained Coelosphaerium. The other algae listed consisted of at least 90% of the genus mentioned. Special effort was always made in the separation of nitrogen-fixing and non-nitrogen-fixing algae because of known nutritional differences.

Since the nutritional patterns of aquatic weeds are not completely understood at present, analyses of aquatic weeds include data obtained by analyses of both the leaves from tips of plants and leaves from more basal sections (at least 8 inches from tips). All analyses of aquatic plants are based upon leaves removed from the stems.

The planktonic algae and aquatic weeds used in the nutritional tests have been identified to the genus level for this report, but samples of many of the critical algal mixtures have been preserved for detailed study if the occasion warrants it.

The algae and aquatic weeds discussed in this report have been collected from either the south shore of Lake Mendota, the west bay of Lake Monona, or from Lake Wingra. The sampling areas are all within about 1 mile of each other. Lake Mendota has an area of 9,700 acres with an average depth of about 13 meters. The watershed of Lake Mendota (142,000 acres) is made up of 73% cropland, 7% woodland, 8% pasture, 5% wetland, and 7% urban. The sampling area on the southern shore (University area) has a rock and broken cement slab shoreline from which Cladophora samples are collected. This area has occasional heavy planktonic algal concentrations, either or both nitrogen-fixing or non-nitrogen-fixing species, during the period from early June to late October. Surface storm sewers drain a small area (about 2 acres) of the University of Wisconsin grounds to the immediate sampling area. The western bay of Lake Monona is separated from Lake Monona by a double (x formation) rail and road fill. The bay has an area of 130 acres and an average depth of about 2.5 meters. The watershed of the bay (812 acres) is entirely urban. Cladophora samples were collected from shoreline rocks at 3 areas (south, west, and north shore) on the bay and also at the mouth of a storm sewer. The bay usually has a bloom of planktonic blue-green algae (Secchi reading of 30-60 cm) a portion of which is usually made up of nitrogen-fixing algae. Data obtained in 1958 indicate that the nutrition composition of the water in the bay is relatively independent of the water in Lake Monona and is assumed to represent an independent shallow lake. Lake Wingra (540 acres, 1.5 meters average depth) is a shallow eutrophic lake which usually supports a heavy growth of phytoplankton: measurements in 1925, 1935, 1942, and 1955 (Eryson et al 1955) indicated that Lake Wingra usually contained two to three times the algal populations of Lakes Mendota and Monona. In more recent years also this lake has usually had a varied phytoplankton population such as to reduce the visibility of the lake bottom to those areas with depths of less than 20-40 cm.

RESULTS

Rain as a source of nutrients for Cladophora

Preliminary tests in June, 1957, with the green alga, Spirogyra sp., growing in a 4.5 acre gravel-lined pond near Madison, Wis. (Salmo Pond) indicated that the rate of NH_4-N utilization of pale, yellowish-green samples of this alga obtained before a rain was 16 µg N/10 mg/hour whereas after a rain (0.4 inches) the alga was bright green and had an NH_4-N utilization rate of 8 µg N/10 mg/hr. Similar effects of rain on the nitrogen nutrition of the Cladophora sp. growing on rocks along the shore of Lake Mendota were also observed in 1957 (Fitzgerald, 1958b).

In order to further evaluate the effect of rain on the nitrogen and phosphorus nutrition of algae, the rates of NH_4-N utilization, the amounts of PO_4-P extracted by boiling water treatments, and alkaline phosphatase activity measurements were made of samples of Cladophora sp. from the southern shore of Lake Mendota (Lake Mendota samples), the shores of the western bay of Lake Monona (Monona Bay samples), and at a storm water sewer outlet (Sewer Outfall samples) in the western bay of Lake Monona. Samples were

collected from the later part of May, 1968, through early September, 1968. The results of NH_4-N utilization rate measurements are presented in Figure 1 as the average rates obtained: each point indicates the average of 6 to 12 samples from 2 to 4 areas on Lake Mendota, 6 to 12 samples from 3 areas on Monona Bay, and 4 to 5 samples from the sewer outlet. The dispersion of data related to each average value presented in Figure 1 can be seen from data from two random dates: June 27, Lake Mendota, mean of 10 samples: 6 µg NH_4-N absorbed/10 mg/hr, range 5 to 7 µg/10 mg/hr. July 15, Lake Mendota, mean of 9 samples: 19 µg NH_4-N/10 mg/hr, range 14 to 26 µg/10 mg/hr. The dispersion was greatest during transition periods. The rainfall data presented are from local U. S. Department of Commerce measurements.

The Lake Mendota samples exhibit a very close correlation between rainfall and a decrease in the rate of NH_4-N utilization, which indicated that after rains the algae had obtained available nitrogen compounds and were less nitrogen-limited at those times of sampling. This was especially true of samples around June 10 and 20, July 15 and 24, August 31 and September 9. Rain had relatively little effect on the rate of NH_4-N absorption of algae which already exhibited a condition of surplus available nitrogen (10 or less µg NH_4-N/10 mg/hr). It might be assumed from these data that after June 5 the main source of available nitrogen for the Cladophora of the surface waters of Lake Mendota was the rain.

In the periods of late May to early June and early August, when the Cladophora appeared to have surplus nitrogen available for the amount of algae present, the presence of epiphytes (diatoms (mainly Rhicosphenia sp.) during the earlier period and mostly blue-green algae (mainly Schizothrix sp.) during the later period) on the Cladophora was considered to be further evidence of the presence of surplus available nitrogen (Fitzgerald 1969). Near June 1 a very heavy Aphanizomenon sp. bloom accumulated and decomposed in the sampling area with no apparent effect on the immediate nitrogen or phosphorus nutrition of the Cladophora. During the later part of July there was a heavy accumulation of a mixed bloom, mainly Lyngbya sp. and Microcystis sp. with some Gloeotrichia sp., but no effect on the nitrogen nutrition of Cladophora was noted. From August 25 to September 9 there was relatively little phytoplankton in the sample area (Secchi reading of 1.5 meters) and the response of the Cladophora to clear, sunny days was good growth (definite increases in length of Cladophora sprigs) and to the rains were drastic decreases in the rate of NH_4-N utilization and changes in color from pale, yellowish-green to bright green.

Rain had similar effects on the rate of NH_4-N utilization by Cladophora samples from Monona Bay and the sewer outfall as it did on Lake Mendota samples. There was only a rough correlation because not as many samples from the bay or sewer outfall were analyzed. Minimal rates of NH_4-N utilization associated with rainfalls occurred in early and late June, mid and late July, and early August. During the later part of August and early September all samples from the bay and sewer outfall had NH_4-N utilization rates of less than 10 µg N/10 mg/hr. In general, Cladophora samples from Monona Bay during August and September exhibited relatively lower NH_4-N utilization rates than samples from Lake Mendota. In late August when Lake Mendota samples were clean and turning pale from nitrogen limitation until rains added available nitrogen, the Monona Bay and sewer outfall samples were heavily coated with epiphytes, mainly diatoms, which further indicated condition of surplus available nitrogen.

Quantitative correlations between the amount of rainfall measured at the local weather station and the effect on the nitrogen nutrition of Cladophora cannot be estimated from the present data because of variations in rainfall between different sampling stations and probable differences in composition of rains (Weibel et al., AWWA 1966).

Analyses to follow the phosphorus nutrition of Cladophora in relation to rainfall are summarized in Figure 2 as the concentrations of PO_4-P extracted per 100 mg of algae by a boiling water bath extraction for 1 hour (Fitzgerald and Nelson 1956). The dispersion of data related to each average value presented in Figure 2 can be seen from data from two random dates: June 27, Lake Mendota, mean of 8 samples: 0.28 mg PO_4-P extracted/100 mg, range 0.24-0.31 mg/100 mg; July 15, Lake Mendota, mean of 8 samples: 0.10 mg PO_4-P/100 mg, range 0.05-0.14 mg/100 mg. The dispersion was greatest during transition periods.

The early spring samples from Lake Mendota exhibited surplus phosphorus. With growth in early June, these values decreased, but the series of rains in June and early August appear to be associated with increased extractable phosphorus. During the peroid July 11 to 31, the Cladophora appeared to be phosphorus limited in that very little PO_4-P could be extracted and relatively high alkaline phosphatase activities were recorded: on July July 31, Cladophora samples had 0.04 mg extractable PO_4-P/100 mg and 1,000 units of an alkaline phosphatase/mg, whereas on August 29, they had 0.17 mg extractable PO_4-P/100 mg and 90 units of alkaline phosphatase/mg. Samples collected August 5 to 7 and all samples after August 19 indicated the presence of surplus available phosphorus. The surplus available phosphorus during the early part of August may be associated with the bloom of Lyngyba and Microcystis which contained surplus phosphorus but there were also 1.4 inches of rain at this time so no definite conclusion can be made. The surplus phosphorus during the later part of August and early September may be due to lack of competition for the phosphorus of the local surface waters because of a relative lack of phytoplankton (Secchi reading of 1.5 meters). In general, decreases in extractable PO_4-P occurred when the algae were in a phase of increasing rates of NH_4-N utilization, a condition expected if the surplus phosphorus of the algae was being used in the growth of the alga.

That the increase in extractable PO_4-P from Cladophora samples collected August 5 to 7 was a sudden change in the phosphorus nutrition of this alga can be confirmed by data obtained with measurements of the alkaline phosphatase activity. It has been shown (Fitzgerald 1958b) that the alkaline phosphatase activity of algae does not suddenly decrease when the algae are placed in an environment with surplus available phosphorus. It is probable that conditions of surplus available phosphorus prevent the further production of alkaline phosphatase so that with growth and dilution of the enzyme, the activity will gradually decrease. Samples of Cladophora collected on July 31 contained 0.04 mg extractable PO_4-P/100 mg and 1,000 units of alkaline phosphatase/mg. Samples from August 5 contained 0.12 mg PO_4-P/100 mg, but still had 1,500 units of alkaline phosphatase/mg. Furthermore, 24 hour incubation of the later sample in laboratory aquaria with surplus available phosphorus caused a further increase in extractable phosphorus, to 0.18 mg PO_4-P/100 mg, with still no decrease in the alkaline phosphatase activity. Therefore, sudden increases in available phosphorus can be differentiated from long periods of relatively high available phosphorus (as in the case of the data from August 29 mentioned in the preceding paragraph) by the fact that the alkaline phosphatase activity will still be high if the algae have only recently had a source of increased available phosphorus, whereas it will be relatively low if the available phosphorus has been in excess for a relatively long period of time.

In contrast to results from Lake Mendota, all but one Cladophora sample from Monona Bay after June 15 indicated the algae were phosphorus limited. There was a significant increase in the extractable PO_4-P from samples in late June which correlates with the very high extractable PO_4-P from sewer outfall samples at that time, samples increasing from 0.04 and 0.03 mg PO_4-P/100 mg on June 20 and 22 to 0.10 mg PO_4-P/100 mg on July 2, then gradually decreasing to 0.045 mg PO_4-P/100 mg on July 10. The levels remained

relatively low for the rest of the summer despite drastic changes in the sewer outfall samples.

The extractable PO_4-P from sewer outfall samples after the rain on June 12 increased from 0.19-0.20 mg PO_4-P/100 mg on June 6, 7 and 12 to 0.30 mg PO_4-P/100 mg on June 14, then fell to 0.12 and 0.14 mg PO_4-P/100 mg on June 22 and 24. The 4 inches of rain after June 24 caused an increase in extractable PO_4-P to 0.19-0.21 mg PO_4-P/100 mg, which later gradually fell to 0.04 mg PO_4-P/100 mg by July 10. Rains on July 15 and 30 caused temporary increases from the usual values of 0.04-0.08 mg PO_4-P/100 mg to 0.14 and 0.28 mg PO_4-P/100 mg. These increases were only temporary, however, since August 5 to 9 the extractable PO_4-P was 0.05 mg PO_4-P/mg or less. The 1 inch rain of August 18 causes another increase in PO_4-P extracted, but all Monona Bay and sewer outfall samples in late August and early September were indicative of phosphorus-limited conditions in contrast to the results obtained from Lake Mendota.

It is evident that available phosphorus did enter Monona Bay in relation to certain rainfalls because of increases in the extractable PO_4-P from the sewer outfall samples. However, only after the very heavy rain in late June did the Monona Bay Cladophora contain what could be considered surplus amounts of phosphorus. Thus, the available phosphorus from the storm sewer could be detected by analyses of algae at the sewer, but algae as little as 50 feet removed were not affected by such sources.

These results illustrate the manner in which these tests can be used for quantitative answers as well as qualitative answers on sources of nutrients. The tests of storm sewer samples indicated both surplus nitrogen and phosphorus were associated with rainfalls, but apparently only nitrogen was added in significantly high enough amounts to affect algae as little as 50 ft from the storm sewer. The amount of phosphorus related to the rains was either not enough to effect distant algae or some factor affected its effective distribution. Therefore, by analyzing samples taken from concentric rings around a possible source of nutrients it should be possible to determine how far away the nutrient source affect both the nitrogen and phosphorus nutrition of existing plant populations.

Sources of nutrients for other attached plants

In May, 1958, when the Lake Mendota Cladophora had NH_4-N absorption rates indicating surplus available nitrogen (10 ug N/10 mg/hr), a sample of Oedogonium sp. from the same area had a similar NH_4-N utilization rate (13 ug N/10 mg/hr). In July, August and September samples of Rhizoclonium sp. (actually a non-branching form of Cladophora) were obtained from weed beds in a depth of 2 to 3 meters off shore from the Cladophora collecting area. On July 29 and August 1 and 2, when Cladophora samples had surplus nitrogen due to recent rains but were phosphorus limited, Rhizoclonium samples were still nitrogen limited (25 to 28 ug N/10 mg/hr). They followed the trend of Cladophora, however, in that by August 19, the NH_4-N utilization rate had decreased to 15 ug N/10 mg/hr. In the period of September 9 to 19 when Cladophora had surplus nitrogen (3 to 10 ug N/10 mg/hr) associated with recent rains, the Rhizoclonium initially was nitrogen-limited (27 ug N/10 mg/hr), but the NH_4-N absorption rate gradually decreased until it was 3 ug N/10 mg/hr on September 19. Further correlations between Cladophora and Rhizoclonium is presented in Figures 3 and 4 and discussed in a later section.

Similarily, in late July and early August, Rhizoclonium samples appeared to be phosphorus-limited (0.08 mg PO_4-P/100 mg), but by September 9 to 10, the amount of extractable PO_4-P was 0.15 mg/100 mg and by September 19, when Cladophora samples had 0.27 mg PO_4-P/100 mg, Rhizoclonium samples had 0.23 mg PO_4-P/100 mg. The

alkaline phosphatase activity of the Rhizoclonium on August 5 was 1,400 units/mg when the extractable PO_4-P was 0.08 mg/100 mg, whereas on September 19, when the extractable PO_4-P was 0.23 mg/100 mg, the enzymatic activity had decreased to 340 units/mg. In general, therefore, the Rhizoclonium from a deeper water area appeared to follow the nutritional trends of the shallow water area Cladophora, except that the effect of recent additions of available nitrogen associated with rainfall did not show up as early after the rainy period as in the case of the surface samples of Cladophora.

Nutritional tests with leaves of the aquatic weed, Myriophyllum sp., collected from Lake Mendota at a depth of 2 to 3 meters (same area from which Rhizoclonium samples were collected) indicated that in early August, when the Cladophora and Rhizoclonium were phosphorus limited, this aquatic weed was also phosphorus limited (tip leaves, 0.06 mg PO_4-P/100 mg; basal leaves, 0.04-0.04 mg PO_4-P/100 mg). During the period of September 9 to 11, when Cladophora and Rhizoclonium samples indicated surplus available phosphorus, the Myriophyllum leaves also had surplus phosphorus (tip leaves, 0.25-0.88 mg PO_4-P/100 mg; basal leaves, 0.14-0.24 mg PO_4-P/100 mg). Thus, this aquatic weed appeared to share the same nutritional patterns as the Cladophora and Rhizoclonium.

As in previous studies (Fitzgerald and Nelson 1966; Fitzgerald 1968a, Fitzgerald 1968b), the effects of incubating plant samples in media known to contain surplus nutrients were measured by placing samples of algae or aquatic weeds in fish aquaria in the laboratory which contained surplus soluble nitrogen and phosphorus compounds from the fish and fish food (Fitzgerald 1969). Samples of Rhizoclonium from Lake Mendota (August 5 and 5), which were phosphorus limited (0.08-0.09 mg PO_4-P extracted/100 mg) and nitrogen limited (23 ug NH_4-N absorbed/10 mg/hr) contained surplus nutrients after only 24 hours incubation in the aquaria (0.30-0.34 mg PO_4-P/100 mg and 10 ug NH_4-N/10 mg/hr). A sample of the aquatic weed, Ceratophyllum sp., which contained only 0.05 mg extractable PO_4-P/100 mg when collected from Lake Wingra on July 31, contained 0.18 mg PO_4-P/100 mg after 48 hours incubation in an aquarium. Short sprigs of Myriophyllum having roots from central areas on the stems when collected from Lake Wingra contained 0.05 to 0.11 mg PO_4-P/100 mg in tiny tip leaves and 0.04 to 0.06 mg PO_4-P/100 mg in larger basal leaves. After 72 hours incubation in an aquarium, tip leaves contained 0.14 mg PO_4-P/100 mg and basal leaves contained 0.08 mg PO_4-P/100 mg. Therefore, the ability of plant samples, which might be limited by the supply of available nitrogen or phosphorus, to absorb and utilize nutrients can be readily determined by such incubation procedures.

Nutrient sources for phytoplankton

The need to separate the various components of algal blooms before nutritional tests are carried out has been mentioned previously (Fitzgerald 1968b). On 12 occasions during the summer of 1968, two or more algal species were separated from mixed blooms and the amounts of extractable PO_4-P or rates of NH_4-N absorption measured. The results of these tests are summarized in Table 1.

In five instances the blooms were separated into two or more nitrogen-fixing algae (Aphanizomenon, Anabaena, or Gloeotrichia). It is interesting to note that in early June when samples from Monona Bay appeared to have surplus available phosphorus, Aphanizomenon contained significantly more phosphorus than Anabaena, whereas, in late June and early July when phosphorus appeared to be less available, there was no significant difference in the amonnts of PO_4-P extracted from these nitrogen-fixing species. It would appear, therefore, that when phosphorus is in excess, some nitrogen-fixing species can contain relatively higher concentrations of extractable PO_4-P than others.

TABLE 1

The phosphorus and nitrogen nutritional status of algae separated from mixed blooms during 1958

Sample		mg PO_4-P Extracted / ug NH_4-N Absorbed per 100 mg	per 10 mg per hr
Monona Bay			
June 7	95% Aphanizomenon	0.29	--
	95% Anabaena	0.13	--
June 17	95% Aphanizomenon	0.41	--
	95% Anabaena	0.28	--
June 22	95% Aphanizomenon	0.07	--
	95% Anabaena	0.08	--
July 3	95% Aphanizomenon	0.07	--
	95% Anabaena	0.10	--
July 3	95% Anabaena	0.09	--
	95% Microcystis	0.13	8
August 21	90% Microcystis	0.23	7
	90% Anabaena	0.09	0
September 3	50% Microcystis and 50% Anabaena	0.24	10
	95% Anabaena	0.09	1
Lake Monona			
July 11	95% Aphanizomenon	0.12	--
	95% Microcystis	0.32	12
July 26	95% Gloeotrichia	0.10	1
	95% Microcystis	0.19	11
Lake Mendota			
June 22	Mixed Gloeotrichia, Aphanizomenon	--	--
	95% Microcystis and Coelosphaerium	0.20	2
July 29	90% Anabaena	0.10	6
	90% Lyngbya	0.17	10
	95% Gloeotrichia	0.12	2
July 30	95% Gloeotrichia	0.13	2

Sample		mg PO_4-P Extracted / µg NH_4-N Absorbed per 100 mg	per 10 mg per hr
Lake Mendota			
	95% Microcystis	0.30	13
August 14	95% Gloeotrichia	0.11	--
	95% Microcystis and Lyngbya	0.23	14
August 15	95% Gloeotrichia	0.12	--
	80% Microcystis	--	15

In all instances when a non-nitrogen-fixing alga (Microcystis or Lyngbya) was separated from nitrogen-fixing species in mixed blooms, very significant differences were found in the amounts of extractable PO_4-P or rates of NH_4-N absorption. The non-nitrogen-fixing algae always had more extractable PO_4-P and higher rates of NH_4-N absorption. Therefore, the importance of separating species of algae when measuring the nutrition of algal blooms must not be under-emphasized.

During May and early June when Cladophora samples had relatively high amounts of extractable PO_4-P, the blue-green algae, Aphanizomenon (3 samples, average 0.33 mg PO_4-P/100 mg), Anabaena (2 samples, average 0.20 mg PO_4-P/100 mg), Gloeotrichia (1 sample, 0.30 mg PO_4-P/100 mg), and Microcystis (1 sample 0.20 mg PO_4-P/100 mg), also had relatively high extractable PO_4-P. A sample of Microcystis from Lake Mendota collected June 22 indicated the presence of surplus available nitrogen (2 ug N/10 mg/hr) at the same time as the Cladophora from the same area also had surplus nitrogen (8 ug N/10 mg/hr). Thus, in the spring there appears to be surplus available nitrogen and phosphorus for the amount of algae present.

After June 20, all nitrogen-fixing blue-green algae collected, Aphanizomenon, Anabaena, and Gloeotrichica, had relatively low extractable PO_4-P (0.13 or less mg PO_4-P/100 mg). In contrast, the non-nitrogen-fixing algae, Microcystis, Coelosphaerium, and Lyngbya, had comparatively high amounts of extractable PO_4-P (13 samples average, 0.28 mg PO_4-P/100 mg).

The transient nature of nutrition within a bloom was demonstrated by changes occurring in a mixed bloom made up mainly of Microcystis and Lyngbya from Lake Mendota in early August. On August 5, the mixture of Microcystis and Lyngbya appeared to have surplus available nitrogen (5 ug NH_4-N absorbed/10 mg/hr) and surplus available phosphorus (0.35 mg PO_4-P extracted/100 mg), whereas, on August 9, the same bloom appeared to be relatively nitrogen-limited (14 ug NH_4-N/10 mg/hr) and contained less extractable PO_4-P (0.25 mg PO_4-P/100 mg). The low NH_4-N absorption rate of August correlates with low NH_4-N absorption rates for Cladophora (3 ug NH_4-N/10 mg/hr) from the same general area. It would appear that the Microcystis-Lyngbya bloom may have grown in this general area and the result was that the algae became relatively nitrogen limited and used up some of their surplus phosphorus. Further evidence of growth of planktonic algae in surface waters is presented in Figure 3 and discussed in a later section.

Correlations between the nutrition of Cladophora and blue-green algae were indicated from data of blooms of Microcystis from Monona Bay which indicated that on July 3, 14 and 24 Microcystis and Cladophora appeared to have surplus available nitrogen, but on July 10 to 11 both Cladophora and Microcystis were comparatively nitrogen limited. The Microcystis and Cladophora of Lake Mendota on July 3 both appeared to have surplus available nitrogen (5 and 10 ug NH_4-N/10 mg/hr, respectively), but by mid-July both appeared to be nitrogen limited (15 or more ug NH_4-N/10 mg/hr). In general, there appeared to some correlation of the nitrogen nutrition of the non-nitrogen-fixing blue-green algae and Cladophora and some correlation of the phosphorus nutrition of the nitrogen-fixing blue-green algae and Cladophora. There was no correlation observed between the nitrogen nutrition of the nitrogen-fixing algae and Cladophora. There was no definite correlation between the phosphorus nutrition of most samples of the non-nitrogen-fixing blue-green algae collected from surface waters and Cladophora during the summer of 1968. As a matter of fact, in late August when Cladophora samples from Monona Bay exhibited phosphorus-limited conditions there was an extremely heavy, viscid mixed bloom of Microcystis and Anabaena coating the Cladophora so thickly that it was difficult to feel the Cladophora when collecting it. This mixed bloom of algae

contained surplus phosphorus (0.17 mg PO_4-P/100 mg) which apparently was localized in the Microcystis (0.23 mg PO_4-P/100 mg) without being available to either the Anabaena (0.09 mg PO_4-P/100 mg) or Cladophora (0.04-0.05 mg PO_4-P/100 mg).

Comparisons of surface and subsurface phytoplankton

Previous reports (Schuette 1918, Lund 1949, Fitzgerald 1968b) have presented data on the relationship between the depth from which algae are collected and their nutrients. Generally, it was assumed that algae from subsurface locations contained higher concentrations of nutrients than surface samples. In order to test this, samples of blue-green algae were collected from Lake Mendota on two occasions in which algae from different depths were compared. The samples compared with surface samples were collected from pipes bringing water from 3 meter and 7 meter depths. The results of nutritional tests with the various samples separated into nitrogen-fixing algae (Gloeotrichia) and non-nitrogen-fixing algae (Microcystis and Lyngbya) are summarized in Table 2.

The amount of extractable PO_4-P from the surface samples of Gloeotrichia (0.11-0.12 mg PO_4-P/100 mg) and the rate of NH_4-N absorbed by the surface samples of the mixture of Microcystis and Lyngbya (14-15 µg NH_4-N/10 mg/hr) indicate that these algae were phosphorus limited and nitrogen limited, respectively. There was no significant difference in the amount of extracted PO_4-P obtained from the surface and 7 meter samples of the mixture of Microcystis and Lyngbya (0.22-0.23 mg PO_4-P/100 mg), thus indicating that phosphorus was probably not a limiting factor for these algae in either location. The fact that the mixture of Microcystis and Lyngbya had decreasing rates of NH_4-N absorption with increasing depths (5 and 0 µg NH_4-N/10 mg/hr) indicates that the subsurface Microcystis and Lyngbya were less nitrogen limited than surface samples (14-15 µg NH_4-N/10 mg/hr). Also, the larger amount of PO_4-P extracted from the subsurface sample of Gloeotrichia indicate it had a surplus supply of available phosphorus at that depth (7 meters, 0.36 mg PO_4-P/100 mg). It would appear that algae from subsurface locations are not as nutrient limited as surface samples of the same type of algae. Perhaps the algae absorb their nutrients and grow in areas of surplus nutrients and when they appear at the surface, they are then affected by the nutrient conditions at the surface at that time: surface Microcystis collected in June and in mid-September had surplus available nitrogen and surface Aphanizomenon, Anabaena and Gloeotrichia collected in May and June had surplus phosphorus, but in mid-summer these types of algae were either nitrogen-limited or phosphorus-limited, respectively.

The reason why blue-green algae come to the surface in lakes is related to their pseudovacuoles, which under pressure can be caused to decrease and the algae will sink. The physiological reason for the increase in pseudovacuoles causing the algae to float to the surface is not known at present. However, an interesting bloom of Microcystis in Lake Mendota on August 20 tends to indicate that normal nitrogen or phosphorus nutrition is probably not involved in the formation or control of the pseudovacuoles. The surface waters of Lake Mendota were particularly free of planktonic algae as late as noon on August 20, but by 2:30 p.m. the surface at mid-lake and the southern shore had a dense floating bloom of pale green Microcystis. Analyses of this alga, which obviously came from a subsurface source, indicated it was neither phosphorus-limited (0.19 mg PO_4-P extracted/100 mg or nitrogen-limited (5 µg NH_4-N absorbed/10 mg/hr). Therefore, it would appear that non-nitrogen-fixing algae do not float only when they run out of available nitrogen and nitrogen-fixing algae do not float only when they run out of phosphorus. Further evidence that normal nitrogen and phosphorus nutrition is not correlated with the floatation of blue-green algae is based on the fact that these species are found to both float or sink in laboratory cultures containing surplus available nutrients.

TABLE 2

Comparison of the nutritional status of surface and subsurface samples of blue-green algae from Lake Mendota

Date	Sample	mg PO_4-P Extracted / ug NH_4-N Absorbed per 100 mg	per 10 mg per hr
August 14, 1968	Surface 95% Gloeotrichia	0.11	--
	" 80% Microcysits + 20% Lyngbya	0.23	14
	7 meters 90% Microcystis + 10% Lyngbya	0.22	0
August 15, 1968	Surface 90% Gloeotrichia	0.12	--
	" 80% Microcystis + 20% Lyngbya	--	15
	3 meters 80% Microcystis + 20% Lyngbya	--	6
	7 meters 90% Gloeotrichia	0.36	--
	" 80% Microcystis + 20% Lyngbya	--	0

Lake overturn as a nutrient source

The fall overturn of a lake takes place when the surface water temperature is reduced to close to that of the bottom water temperature and strong winds cause the first top-to-bottom mixing of the lake since early summer. This mixing of nutrient-rich bottom waters (Mackenthun et al. 1968) with surface waters is a potential source of algal nutrients.

The fall overturn of Lake Mendota took place in early October, 1968. The Microcystis of the mixed plankton at this time was separated (from diatoms and Ceratium sp.) and the rates of NH_4-N absorption and extractable PO_4-P of this planktonic species were compared to those of Cladophora and Rhizoclonium (actually a non-branching form of Cladophora). Microcystis made up the majority of the phytoplankton during the period studied and no other blue-green species were present in significant amounts. The results of NH_4-N absorption tests are summarized in Figure 3 and PO_4-P extractions in Figure 4.

The data of Figure 3 indicate that both Microcystis and Cladophora had surplus available nitrogen (2-3 µg N/10 mg/hr) on September 19 and 20, but with time they became progressively more nitrogen-limited until rates of NH_4-N absorption of 20 µg N/10 mg/hr were obtained during the period September 27 to October 2. The rate of NH_4-N absorption of Rhizoclonium samples on October 2 and 3 also indicated nitrogen-limited conditions (25 µg N/10 mg/hr). By October 4 to 5, the nutrient condition of the algae had changed from a nitrogen-limited condition to surplus available nitrogen (10 or less ug N/10 mg/hr) for all algal samples and this latter condition continued for the next two weeks. Rain occurred (October 5) only after the fall overturn had taken place.

The data summarized in Figure 4 of the extracted PO_4-P from the various algae indicate that on September 19 to 25 the Cladophora samples contained surplus available phosphorus (0.2[illegible]-0.27 mg PO_4-P/100 mg), but by October 1 to 2 the samples were approaching a phosphorus-limited condition (0.10 mg PO_4-P/100 mg). The fall overturn resulted in an increase in the extractable PO_4-P to levels of 0.25 mg/100 mg by October 4 to 5. The samples of Rhizoclonium indicated an increase of extractable PO_4-P of from about 0.2 (October 2 to 5) to 0.2[illegible] mg/100 mg (October 7 to 14). The samples of Microcystis collected September 24 to 27 had 0.16-0.18 mg extractable PO_4-P/100 mg, whereas samples from October 3 to 14 contained 0.2-0.24 mg PO_4-P/100 mg.

These data indicate that the 1968 fall overturn of Lake Mendota provided available nitrogen and phosphorus to the surface water Cladophora and Microcystis and, to a lesser extent, the Rhizoclonium from deeper (2-3 M) depths. The fact that the Microcystis samples exhibited a gradual increasing nitrogen-limited condition correlating with changes in the Cladophora samples of the time indicates that the Microcystis present in the surface waters was probably from a single population and probably not a mixture of surface and deep water Microcystis. Also, these nutritional changes in the Microcystis from September 20 to October 14 indicate that blue-green bloom-forming algae can grow in the surface waters even if their original source may have been more nutrient-rich subsurface depths. The relatively long period (September 20 to October 14) during which Microcystis remained the main blue-green algal species present in the surface waters is unusual in comparison to the succession of various blue-green algal species that occurred during the summer. There is no explanation for the lack of dramatic change in the extractable PO_4-P of the Microcystis during the period of September 24 to October 1 when the extractable PO_4-P of the Cladophora dropped from 0.25 to 0.11 mg PO_4-P/100 mg. The rate of NH_4-N absorption by Microcystis during this period increased from 10 to 20 ug N/10 mg/hr so that the algae appeared to be growing or using their stored nitrogen. It cannot be concluded

that the extractable PO_4-P of Microcystis was already at a minimal concentration on September 24 (0.15 mg PO_4-P/100 mg) because previous data (Fitzgerald and Nelson 1955) had indicated that the extractable PO_4-P of this species could go as low as 0.03-0.06 mg PO_4-P/100 mg when brought into the laboratory and cultured in media lacking only available phosphorus.

It is of interest to point out that, although the fall overturn of Lake Mendota provided available nitrogen and phosphorus to algae in the surface waters, analyses carried out during the same period on algae from Monona Bay and nearby Lake Wingra indicated no increases in nutrient composition. As a matter of fact, in late October the Cladophora of Monona Bay became nitrogen-limited (15-17 ug NH_4-N absorbed/10 mg/hr) after having had surplus nitrogen during the period of August 5 to October 18. This lack of overturn effect in Monona Bay indicates that the water in this bay is not readily affected by recent changes in Lake Monona itself (which is a deep water lake like Lake Mendota and usually has an overturn near the time for Lake Mendota). Therefore, the mixing of the comparatively shallow waters of Monona Bay and Lake Wingra by the very strong fall winds did not appear to increase the available nutrients in the surface waters. These data point out the need for comparative chemical analyses and bioassays for the nutrients available from bottom muds from different water depths and at different periods during the year.

DISCUSSION

The data reported here are based on analyses of particular chemical fractions or enzymatic reactions which reflect limiting or surplus conditions of a nutrient more meaningfully than do total plant analyses. As bioassays in general, the results have a dispersion greater than would be expected in chemical analyses, but in nearly all cases the dispersion obtained from 4 to 12 samples taken at one time was very small compared to the differences between the results obtained before and after a rainfall or other change in nutrient availability. Data from periods of transition, such as during and after the first 12 to 24 hours of a rainfall, exhibited the greatest dispersion.

The dramatic effects of rain were not only apparent from measurements of the rate of NH_4-N utilization, but were readily visible because algae which had been nitrogen-limited for a week or more changed from a pale yellow-green color to bright green immediately after a rainfall. The question of whether this change was due to rain or runoff has not been settled, but it should be pointed out that just as dramatic changes were recorded for the algae from Salmo Pond, which is a 4.5 acre gravel-lined pond with only 8 to 20 feet of drainage area around the pond, as from Monona Bay, which has an urban drainage area of 812 acres.

The forms of nutrients in rainfall may vary in different continental areas and from time to time during the rainfall, but it has been established by Weibel et al. (1956) that rain in two areas of Ohio averaged 0.8 mg inorganic N/liter and 0.05 mg P/liter and by Parker (1958) that rain at St. Louis, Mo., contained as much as 0.02 ug vitamin B_{12}/liter. Chemical analyses and bioassays using the effect of rain on the chlorination of Pseudomonas aeruginosa (Fitzgerald and Der Vartanian 1959) have indicated that rains in the Madison area have contained 0.1 to 0.6 mg NH_4-N/liter. By comparison of analyses of rainfall, runoff, and effects produced on the nutrition of algae meaningful data may be collected concerning the relative sources of algal nutrients.

An attempt has been made to present data which would illustrate some of the factors involved in the availability of nutrients to algae and aquatic weeds. It has been

pointed out that algae maintained in the surface waters of Lake Mendota, such as the filamentous green alga, Cladophora sp., at different times of the year will exhibit both limited or surplus nitrogen or phosphorus for the amount of algae present. The nutrient content of surface waters of a lake, therefore, must be thought of as variables which can be influenced by such factors as the growth of algae removing nutrients, rain or runoff as sources of nutrients, and the fall overturn as a distribution of nutrients from deeper waters.

The phytoplankton of a lake illustrate how nutrient conditions in the surface waters may not be reflected by algae collected as little as 3 meters from the surface. The nutrients available to subsurface algae must be completely different from those at the surface. The planktonic blue-green algae which can float to the surface, therefore, can come from nutrient-rich subsurface waters and grow in the surface waters even if the surface waters at the time are comparatively nutrient-poor. This illustrates the importance of detailed information on samples of water or plankton collected for analyses. One should be very specific to point out that the data described were obtained using algae from a definite depth, whether they were nitrogen-fixing algae, the area of the lake sampled (such as nearness of inlets or sewer outfalls), and the climatic conditions prior to sampling. The influence of a recent rain or the depth of a sample could perhaps cause assays to indicate eutrophic conditions in a lake which for the majority of the summer is really relatively oligotrophic. Neither would it be correct to compare analyses of one lake in mid-summer after a dense growth of algae or aquatic weeds had become established with analyses of another lake which was sampled in the spring. When evaluating the nutrient level of a lake it may be that analyses or bioassays of surface waters should always be contrasted with analyses or bioassays with waters from the thermocline or epilimnion and with waters obtained in the spring before maximum plant production.

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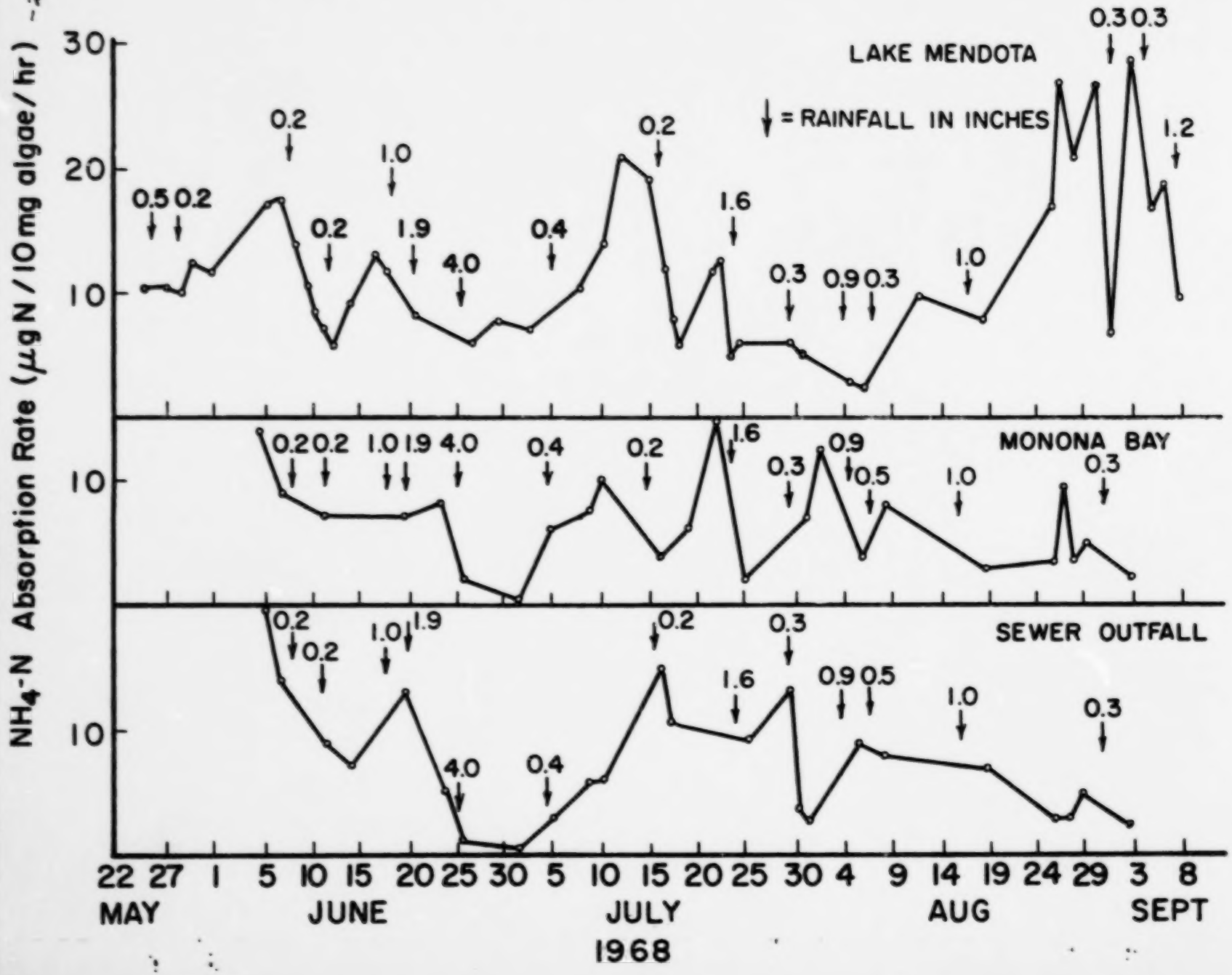
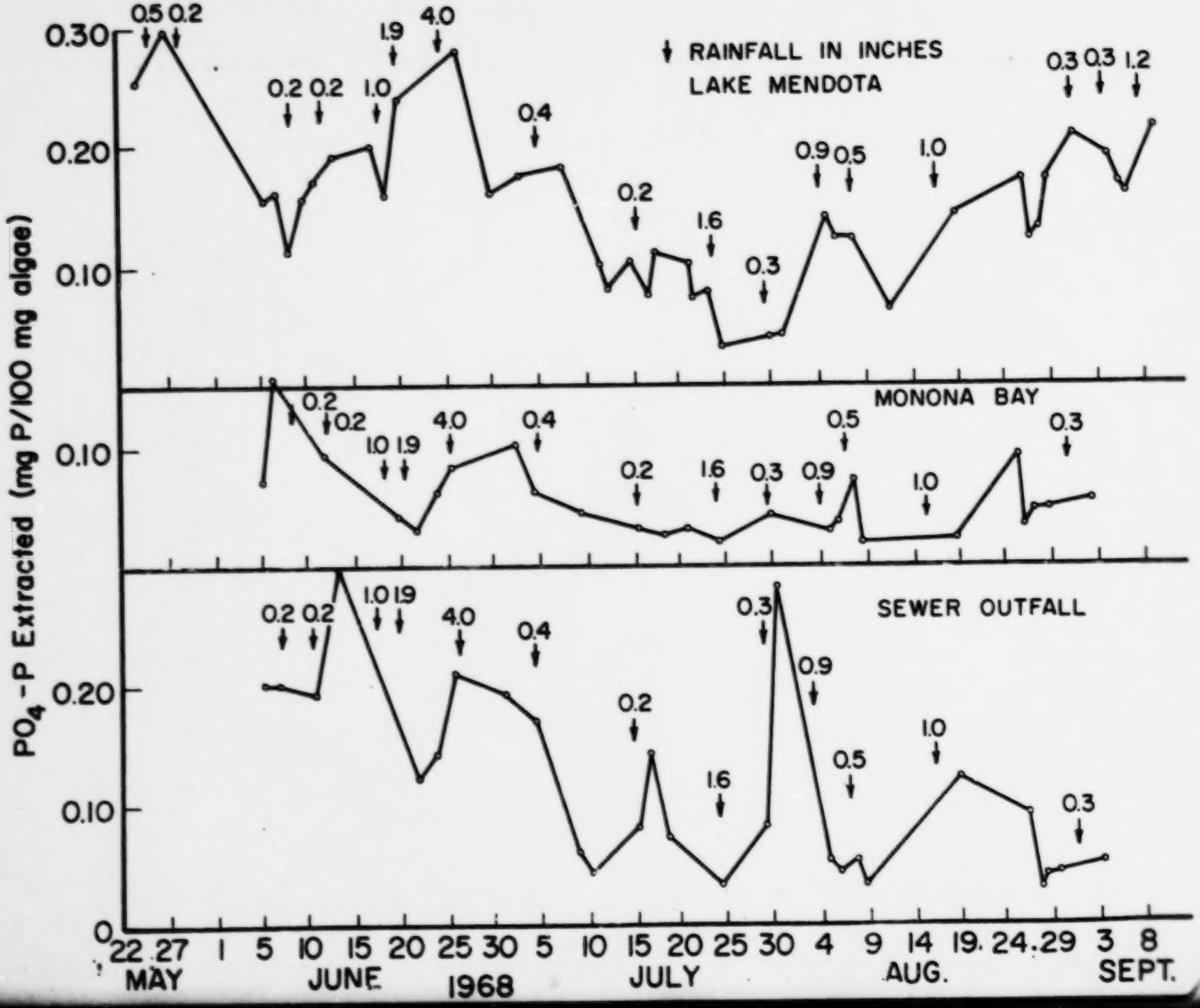
Figure 1. The effects of rains on the NH_4-N absorption rate of Cladophora sp. collected in 1968 from Lake Mendota, Monona Bay, and a storm sewer outfall in Monona Bay.

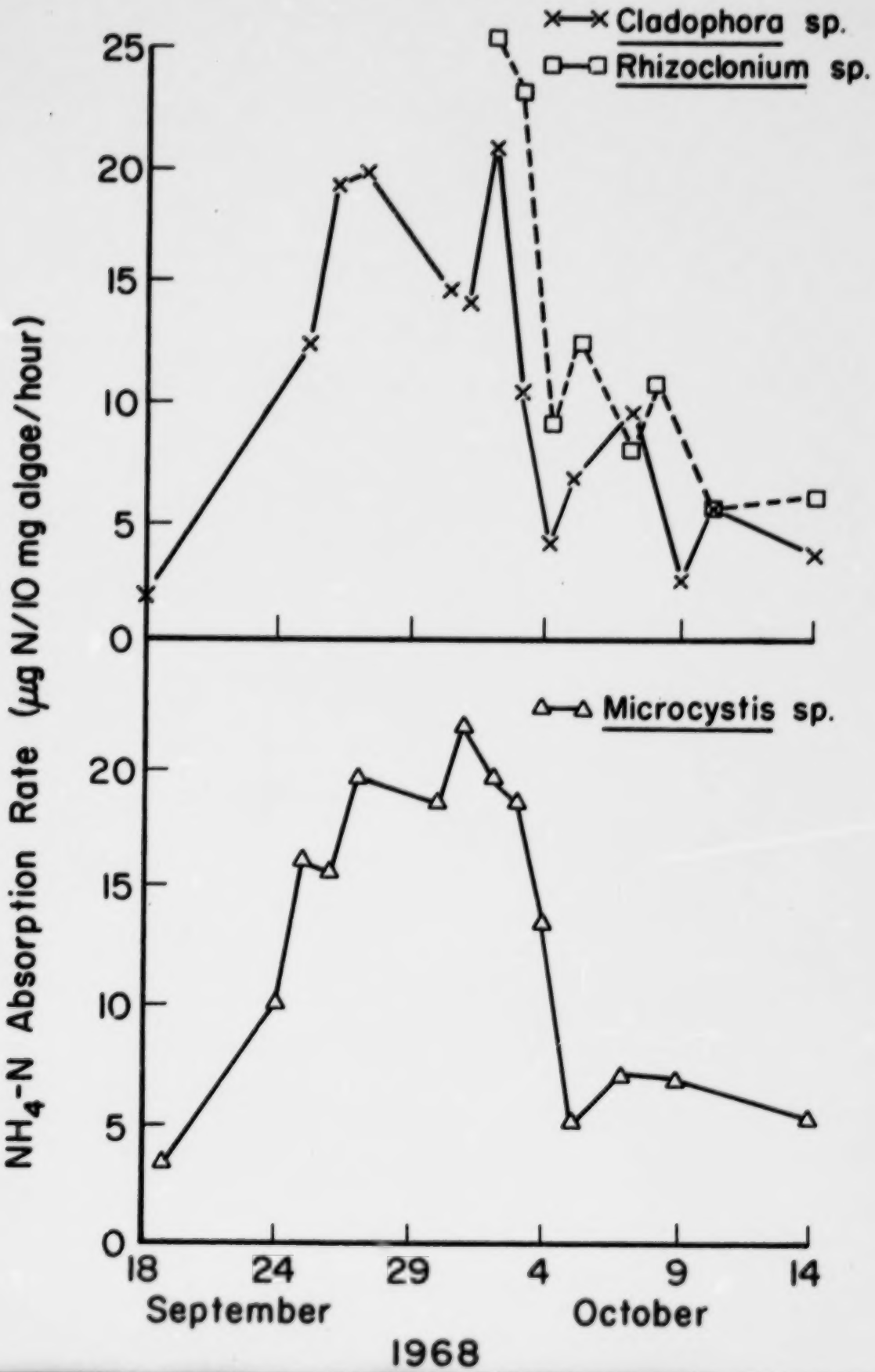
Figure 2. The effects of rains on the PO_4-P extracted from Cladophora sp. collected in 1968 from Lake Mendota, Monona Bay, and a storm sewer outfall in Monona Bay.

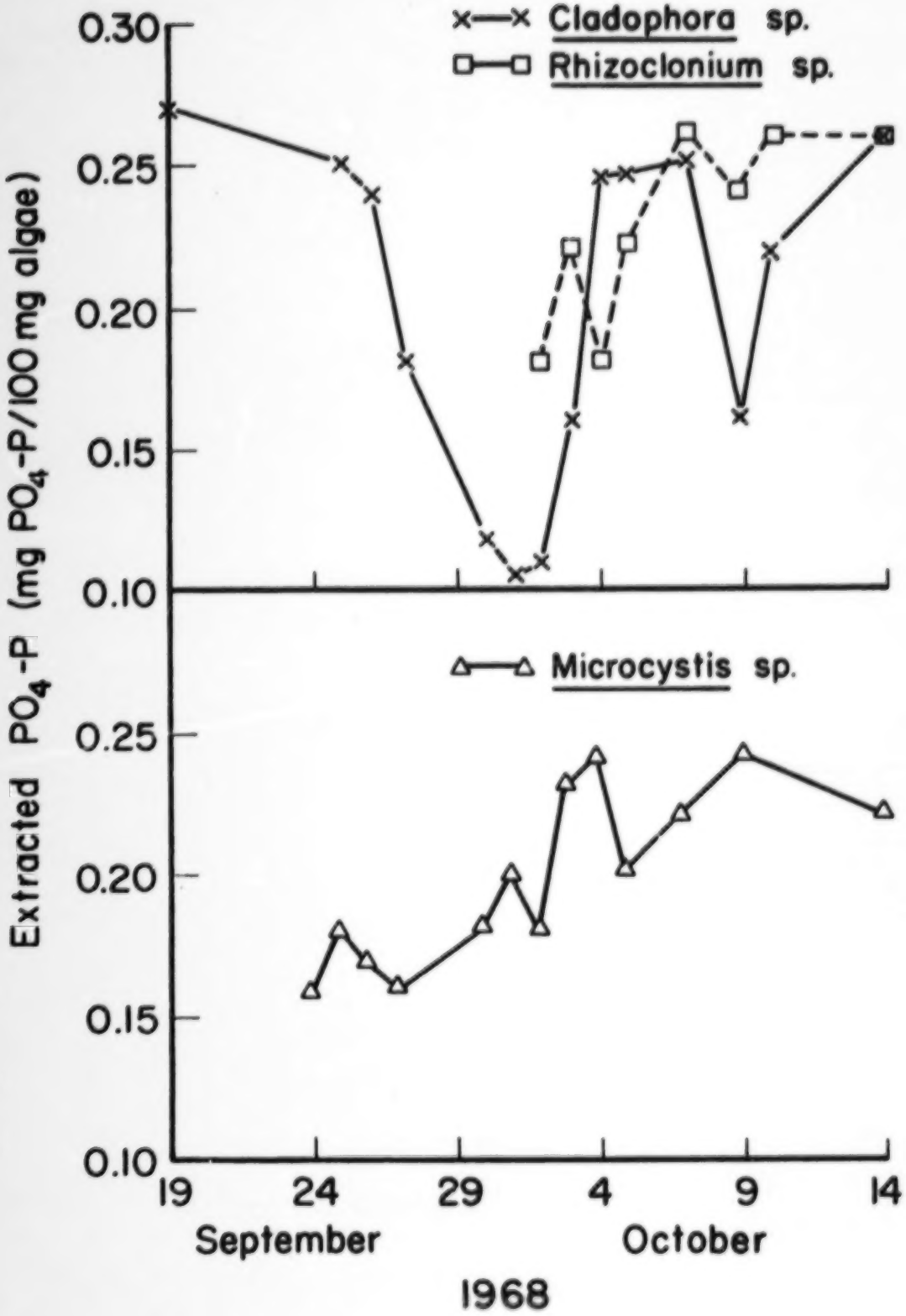
Figure 3. The effects of fall overturn in Lake Mendota on the NH_4-N absorption rate of Cladophora sp., Rhizoclonium (a non-branching Cladophora sp.) and Microcystis sp.

Figure 4. The effects of fall overturn in Lake Mendota on the PO_4-P extracted from Cladophora sp., Rhizoclonium (a non-branching Cladophora sp.), and Microcystis sp.


LAKE MENDOTA
↓ = RAINFALL IN INCHES
MONONA BAY
SEWER OUTFALL
NH_4-N Absorption Rate (μg N / 10 mg algae / hr)
30
20
10
10
10
0.5 0.2 0.2 0.2 1.0 1.9 4.0 0.4 0.2 1.6 0.3 0.9 0.3 1.0 0.3 0.3 1.2
0.2 0.2 1.0 1.9 4.0 0.4 0.2 1.6 0.3 0.9 0.5 1.0 0.3
0.2 0.2 1.0 1.9 4.0 0.4 0.2 1.6 0.3 0.9 0.5 1.0 0.3
22 27 1 5 10 15 20 25 30 5 10 15 20 25 30 4 9 14 19 24 29 3 8
MAY JUNE JULY AUG SEPT
1968


RAINFALL IN INCHES
LAKE MENDOTA
MONONA BAY
SEWER OUTFALL
PO4-P Extracted (mg P/100 mg algae)
0.30
0.20
0.10
0
0.5 0.2
0.2 0.2 1.0 1.9 4.0
0.4
0.2
1.6
0.3
0.9 0.5
1.0
0.3 0.3 1.2
0.2 0.2 1.0 1.9 4.0 0.4 0.2 1.6 0.3 0.9 0.5 1.0 0.3
0.2 0.2 1.0 1.9 4.0 0.4 0.2 1.6 0.3 0.9 0.5 1.0 0.3
22 27 1 5 10 15 20 25 30 5 10 15 20 25 30 4 9 14 19 24 29 3 8
MAY
JUNE
1968
JULY
AUG.
SEPT.


Cladophora sp.
Rhizoclonium sp.
Microcystis sp.
NH4-N Absorption Rate (µg N/10 mg algae/hour)
25
20
15
10
5
0
18
24
29
4
9
14
September
October
1968


Cladophora sp.
Rhizoclonium sp.
Microcystis sp.
Extracted PO4-P (mg PO4-P/100 mg algae)
0.30
0.25
0.20
0.15
0.10
0.25
0.20
0.15
0.10
19
24
29
4
9
14
September
October
1968

Some Factors in the Competition or Antagonism Between Bacteria, Algae, and Aquatic Weeds

SUMMARY

Field observations of changes in the populations of aquatic weeds and phytoplankton have confirmed that aquatic weeds have antagonistic activity against phytoplankton. Nutritional studies in the laboratory indicate that cultures of the aquatic weeds, Myriophyllum sp., Ceratophyllum sp. and duckweed (Lemna minor L.), liquid cultures of barley (Hordeum vulgare L., Dickson variety), and cultures of the filamentous green algae, Cladophora sp. and Pithophora oedogonium (Mont.) Withrock, will remain relatively free of epiphytes or competing phytoplankton if the cultures are nitrogen limited. Field observations of Cladophora sp. have confirmed that the growth of epiphytes on the Cladophora is related to conditions of surplus available nitrogen compounds. It is proposed that this antagonistic activity may be due to a "nitrogen sink" effect in which the aquatic weeds or filamentous green algae prevent the growth of contaminating algae by competition for the limited nitrogen compounds available, but the presence of bacteria-sized organisms which have selective toxicity to certain algae indicates that perhaps multiple factors exist. The ecological implications are discussed of associations of certain algae with bacteria which have selective toxicities to other algae under certain environmental conditions, such as nitrogen-limited growth.

INTRODUCTION

During the course of laboratory nutritional studies with algae and aquatic weeds a number of observations were made which indicated that in an environment in which only a limited amount of nitrogen was available a successful competition could take place between certain filamentous green algae or aquatic weeds and the growth of contaminating or epiphytic algae. The various factors involved in such competition were of interest because of the implied ecological control possible if any of the factors were of a manageable nature. Speculations have been made ever since Hasler and Jones (12) reported on the inhibition of phytoplankton by aquatic weeds as to whether this antagonism was nutritional or antibiotic. We have found some basis for believing that aquatic weed competition with epiphytes is based mainly upon nutritional factors, but have observed reactions in some waters, such as fish aquaria and culture media enriched with sewage plant effluents, which also indicate the presence of antibiotic factors associated with bacteria-sized organisms.

This report will record observations made in laboratory studies and related tests which have been carried out in lakes in support of some of the laboratory findings. It was possible to relate observations of the presence of epiphytes with the nutritional condition of field samples by the use of simple bioassay techniques based on analyses of particular chemical fractions or enzymatic reactions which reflect limiting or surplus conditions of a nutrient more meaningfully than do total plant analyses (4, 5, 8, 9). It is hoped that these observations will stimulate further study so that biologists will have more tools available for the control of obnoxious growths of algae and aquatic weeds.

MATERIALS AND METHODS

The basal algal and aquatic weed culture medium which has been used was Gorham's

medium[1] (13). The basal culture medium used for barley (Hordeum vulgare L., Dickson variety) consisted of (mg/liter): KNO_3, 500; KH_2PO_4, 40; $MgSO_4 \cdot 5H_2O$, 150; $CaCl_2 \cdot 2H_2O$, 150; $FeCl_3$, 3.5; EDTA, 8; and minor elements of Hughes et al. (13).

The chemical analyses for nutrients and total bacteria plate counts were those suggested in Standard Methods (1) except for nitrate-nitrogen which was analyzed for by the chromotropic acid method (17).

The extraction method used to detect the presence of surplus phosphorus in algae or aquatic weeds and the measurement of NH_4-N absorption rates in the dark for the detection of nitrogen-limiting conditions have been described and discussed previously (4, 9).

The field samples of algae and aquatic weeds have been collected from either the south shore of Lake Mendota, the western bay of Lake Monona, or Lake Wingra. Lake Mendota has an area of 9,700 acres with an average depth of about 13 meters. The watershed of Lake Mendota (142,000 acres) is made up of 73% cropland, 7% woodland, 8% pasture, 5% wetland, and 7% urban. The sampling area on the southern shore (University area) has a rock and broken cement slab shoreline from which Cladophora samples are collected. This area has occasional heavy planktonic algal concentrations, either or both nitrogen-fixing or non-nitrogen-fixing species, during the period from early June to late October. Surface storm sewers drain a small area (about 2 acres) of the University of Wisconsin grounds to the immediate sampling area. The western bay of Lake Monona is separated from Lake Monona by a double (x formation) rail and road fill. The bay has an area of 130 acres and an average depth of about 2.5 meters. The watershed of the bay (812 acres) is entirely urban. Cladophora samples were collected from shoreline rocks at 3 areas (south, west, and north shore) on the bay and also at the mouth of a storm sewer. The bay usually has a bloom of planktonic blue-green algae (Secchi reading of 30-60 cm) a portion of which is usually made up of nitrogen-fixing algae. Data obtained in 1958 (8) indicate that the water in the bay is relatively independent of the nutrient composition of the water in Lake Monona and is assumed to represent an independent shallow lake. Lake Wingra (540 acres, 1.5 meters average depth) is a shallow eutrophic lake which usually supports a heavy growth of phytoplankton: measurements in 1925, 1935, 1942 and 1955 (2) indicated that Lake Wingra usually contained two to three times the algal populations of Lakes Mendota and Monona. In more recent years also this lake has usually had a varied phytoplankton population such as to reduce the visibility of the lake bottom to those areas with depths of less than 20-40 cm.

RESULTS

Higher plants versus phytoplankton and epiphytes

Observations made on the phytoplankton and aquatic weeds of Lake Wingra during the summer of 1958 have supported the views of Hasler and Jones (12) and others that there are antagonistic actions between aquatic weeds and algae. In June and July of 1958 Lake Wingra had unusually extensive aquatic weed beds (mainly Myriophyllum sp. with some Ceratophyllum sp.) on all shore areas and the water was quite plankton-free: the bottom was readily visible through 1½ meters. During the later part of July objections by the public to the extensive weed beds caused maintenance crews to cut and remove the weeds from quite extensive areas on the shoreline. By August 5, the water was relatively free of aquatic

[1]Gorham's medium as used contains: (mg/liter): $NaNO_3$, 496; K_2HPO_4, 39; $MgSO_4 \cdot 7H_2O$, 75; $CaCl_2 \cdot 2H_2O$, 36; $Na_2SiO_3 \cdot 9H_2O$, 58; Na_2CO_3, 20; ferric citrate, 6; citric acid, 6; EDTA, 1; pH 8.5

weeds, but the visibility through the water was reduced to about 20 to 40 cm due to the reappearance of the usual mixture of blue-green and green algae. In early September the visibility in Lake Wingra was still low (Secchi reading, 60 cm) but the visibility near the outlet where aquatic weeds had recovered and were quite thick was again such that the bottom was visible in depths of $1\frac{1}{2}$ meters. Thus, there appeared to be evidence that there are antagonistic actions between aquatic weeds and algae in lakes if the conditions are proper.

In contrast to these field observations, when aquatic weeds are placed in culture media in the laboratory, it has been observed that the growth of contaminating algae or epiphytes could be so extensive as to obscure any nutritional response of the aquatic weeds (10). Thus, no antagonistic action was apparent under these conditions. In our laboratory tests we have also found that field collections of filamentous green algae, such as Cladophora or Pithophora, and various aquatic weeds when placed in complete nutrient media soon disappear under extensive growths of contaminating algae. This also took place in liquid cultures of barley (Hordeum vulgare L., Dickson variety) in which the roots are suspended in culture medium. No decrease in contaminants occurred when limiting or surplus phosphorus cultures were compared. However, in all cultures in which available nitrogen was a limiting factor, there appeared to be an antagonistic action between the higher plant and algae. The appearance of cultures of Myriophyllum sp. after three weeks incubation in Gorham's complete medium and in Gorham's medium in which there was no added available nitrogen (-N) are presented in Figure 1 as representative of all the types of plants cultured. In order to summarize the results from a number of types of cultures after two or three weeks of incubation, the cultures were shaken vigorously and 25 ml of the culture filtered through glass pad filters for pictures. The contaminating algae present in cultures of Myriophyllum sp., Ceratophyllum sp. and Cladophora sp. are presented in Figure 2. Similarily, 25 ml samples from barley cultures were filtered for the results presented in Figure 3. Similar results were also obtained with impure cultures of the green alga, Pithophora oedogonium (Mont.) Withrock, and the duckweed, Lemna minor L.

It can be seen that contaminating algae cover Myriophyllum sprigs in complete nutrient medium in as little as three weeks. In contrast, cultures which are nitrogen-limited Gorham's medium have remained green with no visible epiphytes for as long as 13 months in our laboratory. Similarily, the extensive growth of contaminating algae (blue-greens and greens) that took place in cultures of other plants in complete medium is evident. It is interesting to note that almost as much growth of contaminants took place in cultures which were phosphorus limited as in complete medium. In only media lacking nitrogen or both nitrogen and phosphorus did there appear to be any antagonistic or competitive action between higher plants and algae.

It is evident from the data with the barley cultures that the level of nitrogen in the cultures with one-fourth that of the complete medium (17 versus 68 mg N/liter) had considerably less contaminants (mostly Chlorella-like greens) and cultures with only 7 or less mg N/liter had no contaminants. The different dilutions of the phosphorus level (9, 2.3, 0.9 and 0.45 mg P/liter) did not affect the growth of contaminants. After three weeks culture, at which time the barley had four or more leaves on plants in complete medium, although very few contaminants could be detected in the cultures with 7 or less mg N/liter, one could occasionally find small green spots among the roots, or on the floating covers supporting the upper portions of the barley plants, that turned out to be blue-green algae of the Nostocaceae, which are known to be nitrogen-fixers (15, 16). Thus, the antagonistic action of higher plants in liquid cultures against contaminating algae appears to be related to whether or not there is surplus or limited amounts of available nitrogen in the medium.

Further evidence that this antagonism is related to nitrogen nutrition was obtained with cultures of Pithophora in different concentrations of nitrogen and phosphorus. Only in nitrogen-limited cultures would the Pithophora remain clean for three to four week periods. Figure 5-A presents a view of Pithophora filaments (50x) as they appear after two weeks culture in nitrogen-limited Gorham's medium. Figure 5-B is a similar view of Pithophora from cultures in Gorham's complete medium. It is apparent that, although some epiphytes are present in the nitrogen-limited cultures, the Pithophora filaments are almost completely obscured by the growth of a mixture of blue-green and green algal epiphytes in the complete medium. However, if the nitrogen-limited cultures were held for six to eight weeks, or if they were inoculated with known nitrogen-fixing algae, such as Nostoc muscorum (Wis. 1013) or Anabaena flos aquae (Lynbye) Breb. (Ind. 1444), nitrogen-fixing algae were able to grow in these cultures and could compete for space with the Pithophora. Also, if NO_3-N (82 mg N/liter) was added to epiphyte-free Pithophora cultures (four weeks in N-limited medium), within one week after the addition of the available nitrogen the growth of epiphytes was very pronounced and in two weeks time the Pithophora was invisible under the dense growth of epiphytes (blue-green and green algae). Thus, the antagonistic activity of Pithophora in nitrogen-limited culture media must not be due to antibiotic factors or else the nitrogen-fixing blue-green algae could not have grown nor the epiphytes grown after the addition of NO_3-N.

Antagonisms in fish aquaria

The lack of any antibiotic factor in the antagonism between Pithophora and contaminating algae in cultures was quite a shock since this alga had been used for five years in this laboratory to maintain fish aquaria free of contaminating algae (usually diatoms on the glass surfaces and Scenedesmus sp. and Ankistrodesmus sp. in the water). It had been assumed that this observed antagonistic activity was due to some antibiotic factor produced by the Pithophora. When it was found that contaminating algae could grow in cultures of Pithophora when surplus available nitrogen was present, experiments were carried out to determine if this "nitrogen sink" effect of the Pithophora was the only reason for its antagonistic activity in fish aquaria.

Analyses for PO_4-P, NH_4-N, NO_2-N and NO_3-N were made of a number of aquaria used in the culture of guppies (Lebistes sp., veil-tail variety) over a four month period during which normal fish feeding operations were continued. The aquaria used were 30 liter tanks with continuous lighting. Some of the aquaria contained a 6 to 8 cm deep mat of Pithophora and others were treated with algistatic chemicals.

A summary of the results for the periods when water analyses were made is presented in Table 1. It was found that aquaria to which the Pithophora was added remained clear for all four months of the experiment, the fish being visible at all times, whereas those bowls which never contained Pithophora, or from which it was removed, became green in color (Ankistrodesmus sp., Scenedesmus sp. and "Chlorella-like" species; absorbance (500 mu, 1 cm): 0.16-0.18) within seven days, such that the fish were invisible at 15 cm. Aquaria to which 2 mg/liter of the algistatic chemicals, simazine (2-chloro-4, 6-bis(ethylamino)-S-triazine)(18) or diuron (3-(3, 4-dichlorophenyl)-1, 1-dimethylurea) (11), were added remained clear for the four week period they were used. Analyses for NH_4-N and NO_2-N indicated less than significant amounts were ever present in any of the waters (less than 0.3 mg NH_4-N/liter and less than 0.1 mg NO_2-N/liter). There was a tendency for the untreated aquaria to have a slight decrease in PO_4-P concentration during the analytical period. The aquaria that remained clear due to the presence of the Pithophora or algistats had increases in the level of PO_4-P. All aquaria had surplus NO_3-N present at the end of the experiment.

TABLE 1

Changes in fish aquaria with different treatments

Aquaria No.	Treatment	Time Period	Water Color	Supernatant Water Analyses			
				PO_4-P (mg P/liter)		NO_3-N (mg N/liter)	
				Initial	Final	Initial	Final
3*	None	1/2 - 3/18	Green	2.0	1.1	5.5	1.9
8*	Pithophora	1/2 - 3/18	Clear	0.9	1.4	2.4	14.
1	Pithophora	1/2 - 2/26	Clear	0.04	0.07	0.8	1.4
1	None	3/4 - 3/18	Green	0.05	0	1.6	7.5
2	Diuron (2 mg/l)	2/19 - 3/18	Clear	0	0.16	0.3	6.6
6	Simazine (2 mg/l)	2/19 - 3/18	Clear	0.08	0.56	1.4	22.

*Aquaria No. 3 and 8 had been in operation for 2 months prior to this period.

It would appear, therefore, that some factor other than a "nitrogen sink" was involved in the antagonistic activity of Pithophora towards contaminating algae in fish aquaria.

Antagonisms between bacteria and algae

Preliminary tests to determine what other factor could be involved in the antagonistic activity of Pithophora involved the use of subcultures with aquaria water. Samples of supernatant water from aquaria containing Pithophora were placed in Erlenmeyer flasks in the algal culture room, to some additional nitrogen (20 mg NO_3-N/liter) and phosphorus (3 mg PO_4-P/liter) were added, and inoculations made of either Chlorella pyrenoidosa (Wis. 2005) or the mixture of green algae from untreated aquaria such that the initial cell counts were approximately 300,000 cells/ml. It was found that the growth of Chlorella in these cultures was erratic, sometimes growing up very rapidly, but then decreasing overnight. The growth of Ankistrodesmus sp. in flasks inoculated with aquaria phytoplankton was usually quite consistent and related to added nutrients. Therefore, similar tests were carried out with Chlorella after the aquaria water was pretreated in various ways. Filtration of the water through Whatman No. 42 filter paper or glass pad filters to remove possible animal contaminants had no effect on the inhibition of growth of added Chlorella cells. However, sterilizing the water by membrane filtration or autoclaving allowed the added Chlorella to survive and grow. Subcultures from inhibited flasks to freshly inoculated Gorham's medium further indicated that there was an organism present which could pass through filter paper or glass fiber pads but not through 0.45 u membrane filters which inhibited Chlorella but not Ankistrodesmus sp. Therefore, there appears to be a complex of factors involving bacteria-sized organisms associated with a differential antagonism between the filamentous green alga, Pithophora, and the phytoplankton or epiphytes of fish aquaria.

A similar antagonistic activity was found to occur when attempts were made to culture the blue-green alga, Microcystis aeruginosa (Wis. 1036), in various sewage treatment plant effluents. In a series of 25 samples tested over a five month period, 19 samples did not support the growth of added Microcystis (1,500,000 cells/ml) when tested as untreated samples whereas Chlorella was found to be able to grow in all 25 samples. When the 19 samples which prevented the growth of Microcystis were sterilized by autoclaving or membrane filtration of the samples before inoculation the alga was found to grow (absorbancies (500 mμ, 1 cm) of 0.2-0.4 after 9 to 11 days).

Further tests were made to attempt to characterize this observed antagonistic action. Samples from an Ely, Minnesota, and Madison, Wisconsin, sewage treatment plant were tested. The six samples from Ely were all final effluents from the trickling filter treatment plant and the four samples from Madison were either trickling filter effluent, activated sludge (aeration) effluent, or final mixed effluent. The treatments given to the various samples are presented in Table 2. Sterilization was done by autoclaving (15 lbs, 15 minutes) or by membrane filtration (0.45 μ). The membrane filtration was carried out at both the normal pH 7 to 8 of the samples and at pH 4 in order to prevent the removal of possible toxicants, such as copper, which might not be completely soluble at the higher pH values (5, 7). Partial chlorination of some of the samples was carried out by adding 10 mg/liter of free, available chlorine and dechlorination with sodium sulfite after 30 minutes. This treatment reduced the bacterial numbers to about 100 colonies/ml. Sonication by using the maximum power setting of a Blackstone SP-2 sonicator for 1 minute was used as a differential killing agent. Total bacterial plate counts before and after sonication indicated no significant loss in the total numbers. The addition of the chelating agent EDTA (5 mg/liter was used to reduce the toxicity of any heavy metal toxicants (5, 14). The results of these tests are summarized in Table 2 as the average growth attained (absorbances, 500 mμ, 1 cm) by the added Microcystis after 9 to 11 days incubation.

TABLE 2

The effect of treatments of sewage plant effluents on the growth of Microcystis aeruginosa (Wis. 1036)

	Growth attained as optical densities (500 mμ, 1 cm) after 9-11 days									
	Ely, Minnesota Samples						Madison, Wisconsin Samples			
Treatment	5-301 0535	5-304 1800	7-305 1400	8-C 220	9-311 1500	11-C 270	Filter 11/10	Aeration 11/10	Final 11/10	Final 11/27
Raw	0.01	0.01	0.01	0.01	0.01	0	0.01	0.01	0	0
Autoclaved	.10	.19	.14	.25	.26	--	.16	.14	0.14	0.60
Membrane filtered[1]	.02	.14	.12	.19	.22	--	.15	.07	.16	.24
Membrane filtered[1] at pH 4	.02	.10	.15	--	--	--	.17	.16	.22	--
Chlorinated[2]	.06	.10	.13	--	--	0.43	.10	.16	.15	.49
Sonicated[3]	--	--	--	--	--	.35	--	--	--	.27
EDTA added[4]	.01	.01	.01	.01	.01	0	0	.02	.01	--

[1] Membrane filtration: Millipore HA (0.45 μ); filtered at pH 7-8 or after adj. to pH 4.

[2] Chlorination: 30 min. treatment with initial conc. of 10 mg/l, dechlorinated with sodium sulfite.

[3] Sonication: 35 ml/50 ml Erl. flask with max. power Blackstone SP-2 for 1 minute.

[4] EDTA: 5 mg EDTA/liter.

The data indicate that all samples sterilized by autoclaving would allow the added Microcystis to grow as compared to little or no growth in raw or untreated samples. There was no consistent difference in results obtained by membrane filtration at the normal pH values or after adjusting to pH 4. Therefore, the filtration was not removing a toxicant which was relatively insoluble in the neutral or alkaline range and soluble at pH 4, such as copper. What was removed by filtration, however, was the cause of the inhibition of the growth of Microcystis. The partial chlorination and the sonication treatments caused the destruction of the inhibitory factor even though they only brought about, at most, a selective kill of some bacteria present. The bacteria remaining after these partial kills apparently had no adverse effects on the growth of the added Microcystis. Further evidence that heavy metal toxicity was not involved in the inhibition of Microcystis was obtained when the Microcystis failed to grow in the EDTA-treated sewage samples.

Further tests with filtration techniques indicated that the inhibitory factor in the sewages was not removed by filtration through Whatman No. 42 filter paper or glass fiber pads (Reeve Angel No. 202). The conclusion that the inhibitory factor was bacterial was enhanced by the nature of the inhibition. In most instances, the added Microcystis grew up in raw samples of sewage effluents but the algal mat on the bottom of the flasks would then take on a holey appearance and completely disappear in a few days. This is demonstrated in Figure 4 which is a picture of the growth of Microcystis in Gorham's medium and in this medium plus the addition of 1 cc or 1 drop of final effluent from the Madison sewage treatment plant. It can be seen that at the stage presented the algal mat on the bottom of the flasks with the added sewage effluent is partially decomposed. In two more days the Microcystis had disappeared and the flasks with sewage effluent were clear. Further subcultures from these flasks were also inhibitory to Microcystis cultures.

Field observations of antagonism between Cladophora and epiphytes

The data presented thus far have indicated that there are antagonistic activities exhibited against epiphytic algae in nitrogen-limited cultures of some filamentous green algae, aquatic weeds, and barley and selective antagonistic factors associated with bacteria-sized organisms in fish aquaria and sewage plant effluents. In order to expand the information obtained by these observations in the laboratory, some of the results were checked with observations of algae in the Madison lakes. Routine analyses have been made of certain algae during the summer of 1958 in order to determine some of the factors influencing the nitrogen and phosphorus nutrition of the algae (8).

The rate of NH_4-N absorption in the dark by algae was used to determine if the algae had surplus or limiting available nitrogen at the time of sampling. Algae which are nitrogen limited can absorb NH_4-N in the dark four to five times faster than algae with adequate available nitrogen (4). Algae that are phosphorus limited contain little or no extractable PO_4-P (1 hour boiling water bath) but have relatively high alkaline phosphatase activities (9). It was found that analyses of samples of Cladophora sp. collected from the shores of Lake Mendota or the western bay of Lake Monona (Monona Bay) indicated that this alga was nutrient limited at certain times and had surplus nutrients at others. In the early spring the Cladophora of Lake Mendota had surplus available nitrogen (10 ug NH_4-N absorbed/10 mg algae/hr) and were covered with epiphytes, mainly the diatoms Phoicosphenia sp., Melosira sp. and Nitzschia sp. The Cladophora became nitrogen limited in early and mid-June, mid-July and late August, the NH_4-N absorption rates being 14 to 27 ug N/10 mg/hr. During these times the Cladophora was relatively free of any epiphytes. During the later part of July and early August the Cladophora had an extended period in which it had surplus available nitrogen (5 or less ug NH_4-N absorbed/10 mg/hr) due to considerable rain at this

time and samples were coated with epiphytic blue-green algae (mainly Schizothrix sp.). Figure 5-C is a photograph (50 X) of Lake Mendota Cladophora collected on July 15, 1958, at a time when it was relatively free of epiphytes. Figure 5-D is a view of Lake Mendota Cladophora collected on August 5, 1958, when it was well coated with epiphytic blue-green algae. At the time in spring when the Cladophora was coated with epiphytes the Cladophora had surplus available phosphorus (0.25-0.30 mg PO_4-P extracted/100 mg algae), but in late July when they also had epiphytes the Cladophora was relatively phosphorus-limited (0.03-0.03 mg PO_4-P extracted/100 mg). Thus, the presence of epiphytes on the Cladophora of Lake Mendota appears to be associated with periods when the algae had surplus available nitrogen and not related to their phosphorus nutrition.

The Cladophora samples from Monona Bay during most of the summer had surplus available nitrogen, many samples having NH_4-N absorption rates of 10 or less ug N/10 mg/hr, but were relatively phosphorus-limited (0.08 or less mg PO_4-P extracted/100 mg). Nearly all samples collected during this period of surplus available nitrogen were heavily coated with epiphytic diatoms, mainly Rhoicoshenia sp., Melosira sp., and Nitzschia sp. Figure 5-E (50 X) and Figure 5-F (150 X) are photographs of Cladophora from Monona Bay on August 29 indicating the manner in which they were coated with epiphytic diatoms at a time when the Cladophora of Lake Mendota was relatively free of epiphytes.

In late October, 1958, the Cladophora of Monona Bay grew under conditions in which it was relatively nitrogen limited as compared to earlier conditions: NH_4-N absorption rates for the period, August 5 to October 18, were consistently less than 10 ug N/10 mg/hr, whereas NH_4-N absorption rates on October 24 and 25 were 15-17 ug N/10 mg/hr. The appearance of the Cladophora also changed at this time: during the period, August 5 to October 18, the Cladophora was brown in color due to epiphytic diatoms, whereas on October 24 and 25 the basal portions were still brownish in color due to epiphytic diatoms, but the younger tip areas were light green and relatively free of epiphytes. Also, Cladophora samples collected on October 24 and 25 at the openings of two storm sewers in Monona Bay were shown to have surplus available nitrogen (5-7 ug NH_4-N absorbed/10 mg/hr) associated with recent rains and to be completely coated with a brown covering of epiphytic diatoms. From these observations it might be assumed that the presence of dense growths of epiphytes on Cladophora could be used as a preliminary indication of the presence of surplus available nitrogen in the environment.

Lack of sharing of algal nutrients

It was of interest to check the data that had been accumulated from field observations and nutritional tests (8) to determine if there was any evidence of the sharing of nutrients by one type of algae with another. In late August when the Cladophora of Monona Bay exhibited phosphorus-limited conditions there was an extremely thick, viscid mixed bloom of Microcystis and Anabaena coating the Cladophora so thickly that it was difficult to feel the Cladophora when picking it from the rocks. This mixed bloom of algae contained surplus phosphorus which apparently was localized in the Microcystis (0.23 mg PO_4-P extracted/100 mg algae) without being available to either the Anabaena (0.09 mg PO_4-P/100 mg) or Cladophora (0.04-0.05 mg PO_4-P/100 mg). Thus, this intimate mixture of algae was not able to share the surplus phosphorus of the Microcystis.

Further evidence for the lack of sharing of algal nutrients was obtained by separating the nitrogen-fixing species of algae from non-nitrogen-fixing species when they occurred in mixed blooms. On 12 occasions during the summer of 1958, two or more algal species were separated from mixed blooms and the amount of extractable PO_4-P or rates of NH_4-N

absorption measured (8). In all instances when a non-nitrogen-fixing alga (Microcystis sp. or Lyngbya sp.) was separated from nitrogen-fixing algae (Aphanizomenon sp., Anabaena sp. or Gloeotrichia sp.) during the mid-summer period when the surface waters were shown to be nutrient limiting very significant differences were found in the amounts of extractable PO_4-P or rates of NH_4-N absorption. The non-nitrogen-fixing algae always had more extractable PO_4-P and higher rates of NH_4-N absorption, thus indicating that they had surplus phosphorus but were probably nitrogen limited. The nitrogen-fixing algae were never nitrogen limited, but were phosphorus limited. Thus, neither the nitrogen of nitrogen-fixing algae nor the phosphorus of the non-nitrogen-fixing algae was available to each other.

DISCUSSION

The observations made on changes in the aquatic weed and phytoplankton populations of Lake Wingra during 1968 have supported the views of Hasler and Jones (12) and others that there are antagonistic actions between aquatic weeds and phytoplankton. That the observed antagonism in Lake Wingra was not related to environmental factors only in the lake basin, but to more general regional factors this year is supported by observations made in 1968 of another local shallow eutrophic lake (2), Lake Kegonsa, in which one bay supported an unusually heavy aquatic weed infestation to the exclusion of the usual heavy phytoplankton blooms. The factors involved in determining whether aquatic weeds or phytoplankton will dominate the plant population of an aquatic environment are of interest because of the implied ecological control possible.

Laboratory studies of the antagonism between aquatic weeds and phytoplankton have always been difficult to carry out because of the dense growths of epiphytes or contaminating phytoplankton which completely cover the aquatic weeds when attempts are made to cultivate field collections in laboratory culture media. Gerloff and Krombholz (10) circumvented this by using sterilized seeds for their sources of aquatic weeds and using sterile nutrient media for nutritional tests. The observations of aquatic weed and filamentous green algal cultures which indicated that the antagonism observed in lakes would also take place in laboratory cultures if nitrogen was a limiting nutrient in the media but was not related to phosphorus nutrition was a good start in understanding some of the factors involved. The data accumulated tended to indicate that the competition must be related to either a "nitrogen sink" phenomenon in which the higher plants could prevent the growth of phytoplankton or epiphytes by competition for the limited amount of available nitrogen or that there was an antibiotic factor produced by the higher plants when they were stressed by a limiting-nitrogen environment. Whitton (19) has reviewed the production of some extracellular products by algae and pointed out that some of these products are more active at certain stages of growth than at other stages of growth.

The results with the barley and Pithophora cultures which indicated that nitrogen-fixing blue-green algae could grow in nitrogen-limited cultures which had appeared to be antagonistic to other algae seems to rule out the possibility of the antagonism in nitrogen-limiting environments being due to antibiotic factors produced by the higher plants.

The fact that surplus NO_3-N was consistently found in fish aquaria which were relatively free of contaminating diatoms and green and blue-green algae because of the presence of Pithophora oedogonium (Mont.) Withrock made it apparent that more than a "nitrogen-sink" was involved in such antagonistic action. The species of nitrogen, NH_4, NO_2, or NO_3, or the relative concentrations that are present may be of importance, but in

Gorham's culture medium containing 82 mg NO_3-N/liter Pithophora was not able to prevent the growth of epiphytes whereas it did in aquaria with NO_3-N concentrations of 1.4 to 14 mg N/liter. Therefore, the presence of bacteria-sized organisms in the supernatant of Pithophora-containing fish aquaria which were antagonistic to the very persistent laboratory algal weed, Chlorella pyrenoidosa (Wis. 2005) was very interesting. This bacteria-sized organism may not be of consequence in the inhibition by Pithophora of the normal contaminating flora of aquaria, diatoms, Scenedesmus sp. and especially, Ankistrodesmus sp., but points out how bacteria could play a role in such antagonisms.

The role of bacteria as possible factors in the control of dominance in phytoplankton blooms in sewage ponds and lakes (3) is also suggested from the data on the bacteria-sized inhibitor which prevented the growth of Microcystis aeruginosa (Wis. 1035) in sewage plant effluents, but did not affect the growth of Chlorella. It could be that each species of algae could support the growth of certain bacteria under specific nutritional conditions, such as nitrogen-limited growth. These bacteria may not affect the algae they were associated with, but might inhibit the growth of competitive algae. The fact that mixtures of algae occur at times and at other times some of these same species occur in almost unialgal condition, especially blue-green phytoplankton in eutrophic lakes, seems to support the idea that the antagonistic factors involved are only present under certain conditions. Furthermore, it has been demonstrated (3) that the factors which allow blue-green algae to exist as unialgal populations in some lakes is very labile and disappears when the dominant blue-green species is selectively killed with toxic chemicals so that within four days a new species can reach effective dominance. Thus, the environmental conditions determine whether one species will be able to dominate all others because changes in dominance can be artifically brought about but each new species becomes as successful as the preceding species.

It could be that the filamentous green algae support a characteristic bacterial flora which inhibit the growth of epiphytes or phytoplankton only when the filamentous green algae are growing under nitrogen-limited conditions. The observations made in Lake Mendota and in Monona Bay that the Cladophora sp. present is free of epiphytes when it is nitrogen-limited supports such an assumption, but a great deal of further work would be required to prove it.

The theory describing mixed algal blooms or the association of filamentous green algae or aquatic plants and epiphytes as being associations formed because of the sharing of surplus nutrients cannot be supported by the data presented. There was no evidence of effective sharing of the surplus phosphorus of Microcystis sp. or Lyngbya sp. with phosphorus-limited Cladophora or the phosphorus-limited nitrogen-fixing algae, Anabaena sp., Gloeotrichia sp., or Aphanizomenon sp. and no evidence of effective sharing of the nitrogen of the nitrogen-fixing blue-green algae with non-nitrogen fixing blue-green algae.

CONCLUSIONS

Observations made of laboratory cultures and field populations of aquatic weeds and filamentous green algae and the presence of epiphytes or phytoplankton have confirmed that antagonistic activity exists between such populations when the aquatic weeds or filamentous green algae are nitrogen-limited. It can be concluded that the presence of dense growths of epiphytes on populations of Cladophora could be used as evidence that the local aquatic environment had a surplus supply of available nitrogen for the requirements of the algal population.

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Figure 1. Comparison of epiphytic growths of algae in Myriophyllum sp. cultures after 3 weeks incubation - complete medium versus nitrogen-limited medium.

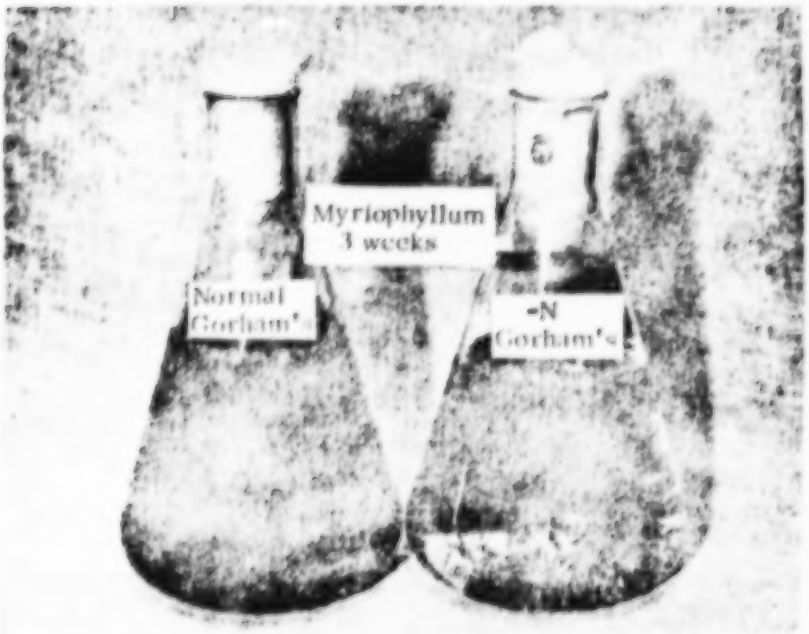

Figure 2. The effect of the medium composition on the growth of contaminating algae in cultures of aquatic weeds and Cladophora sp.

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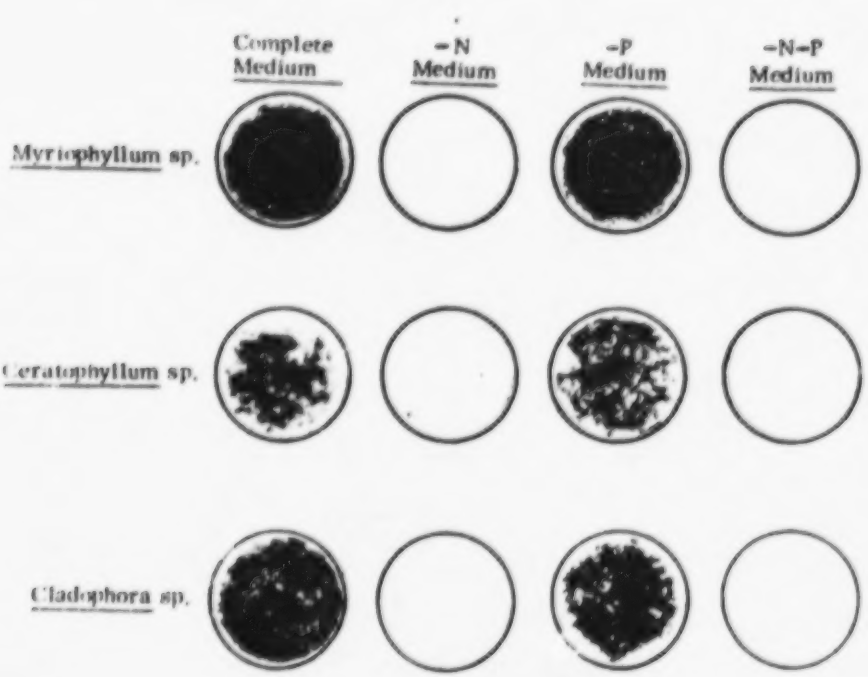

Figure 3. The effect of the medium composition on the growth of contamination algae in liquid barley cultures.

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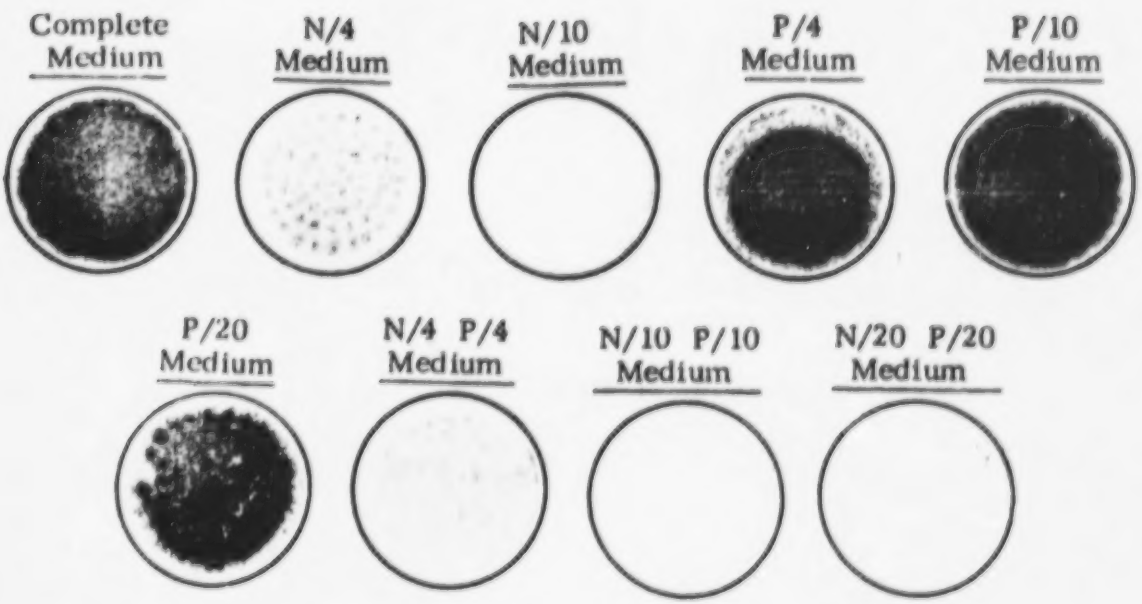

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Figure 4. The effect of the addition of Madison sewage treatment plant effluent on the growth of Microcystis aeruginosa (Wis. 1036) in Gorham's medium.

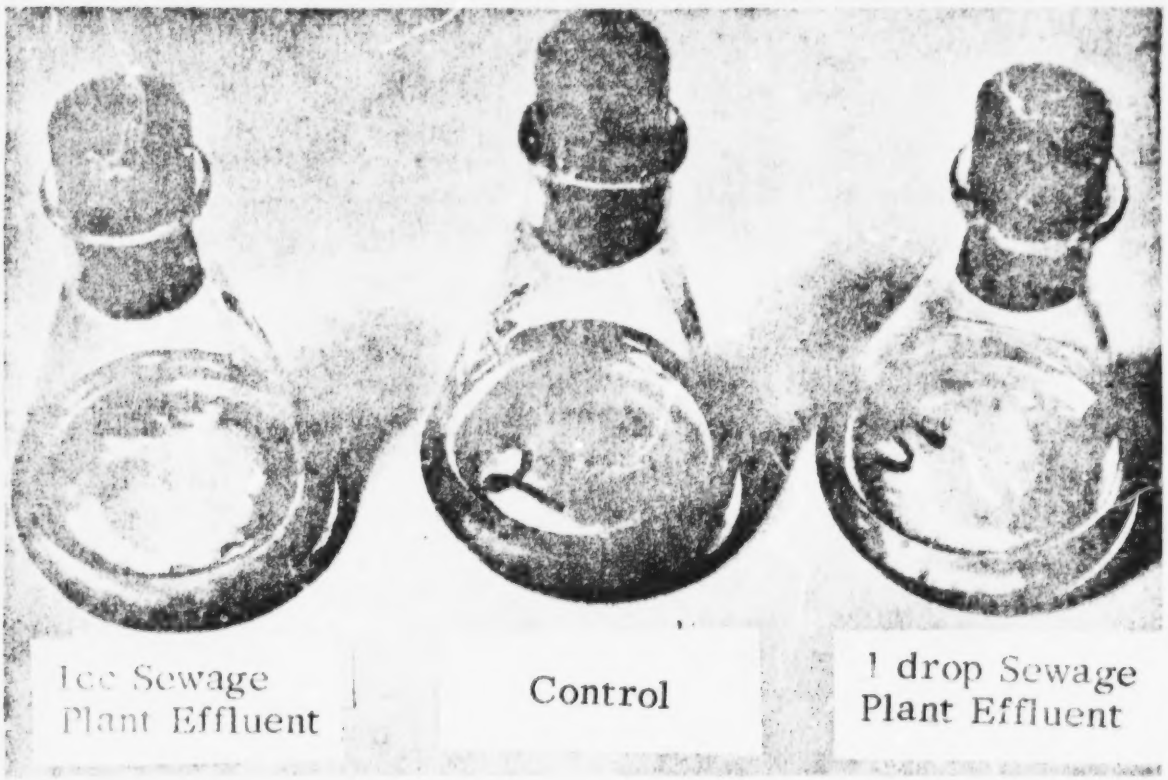

Figure 5. Relationship between nitrogen nutrition and the growth of epiphytic algae.

A. Pithophora oedogonium (Mont.) Withrock cultured 2 weeks in nitrogen-limited Gorham's medium (50x).

B. Pithophora oedogonium (Mont.) Withrock cultured 2 weeks in complete Gorham's medium (50x).

C. Cladophora sp. from Lake Mendota shore, July 15, 1968 (20 µg NH_4-N absorbed/10mg/hr) (50x).

D. Cladophora sp. from Lake Mendota shore, August 5, 1968 (4 µg NH_4-N absorbed/10 mg/hr) (blue-green algal epiphytes) (50x).

E. Cladophora sp. from Monona Bay shore, August 29, 1968 (4 µg NH_4-N absorbed/10 mg/hr) (diatom epiphytes) (50x).

F. Cladophora sp. from Monona Bay shore, August 29, 1968 (150x).

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